CARCASS SHAPE AND MEAT EATING QUALITY IN SHEEP: OPPORTUNITIES FOR GENETIC IMPROVEMENT USING COMPUTED TOMOGRAPHY

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ABSTRACT

This thesis reports on an investigation of the association between muscularity and meat quality in Scottish Blackface (SBF) and Texel (TEX) lambs, and the *in vivo* assessments of these traits using X-ray computed tomography (CT) with a view to their possible inclusion in breeding programmes. The objectives of this work were: (i) to develop comprehensive *in vivo* assessments of muscularity using spiral CT scans; (ii) examine the relationship of the new muscularity indices with carcass and eating quality; (iii) explore the associations among CT assessments of carcass composition, muscularity and muscle density, and (iv) investigate the possibility of limiting the antagonism between selection for reduced fatness and maintaining eating quality by introducing a CT predictor of intramuscular fat (IMF) as an additional selection criterion for the breeding programmes using CT.

The calculation of muscularity indices requires the measurement of the muscle mass and skeletal dimension of the regions of interest. Priority was given to the hind leg (HL) and lumbar region (LR), where high priced cuts are located. The utilisation of new novel imaging technology called spiral CT scanning, which captures detailed information on any specific region, was explored. An algorithm to automatically segment the spiral CT scans (SCTS), and procedures to assess the real dimensions of skeletal structures, were developed. Compared to previous CT muscularity measurements, the accuracy was much higher with the new index in the HL (correlations with equivalent indices bases on dissection of 0.89 *vs* 0.51). The accurate measurement of femur length by CT used in the calculation of the new HL index made an important contribution to the higher accuracy of the index. The improvement in accuracy was smaller for the LR (0.55 *vs* 0.44).

The association of CT muscularity indices and carcass traits by dissection in both breeds showed that improved muscularity is not phenotypically correlated with detrimental effects on other carcass quality traits. The correlation coefficients, after adjusting for carcass weight, were positive with meat yield and low and negative, or close to zero, with fatness. This is particularly relevant for the terminal sire breeds, in which the economically important traits included in breeding programmes tend to be carcass composition traits. In the case of SBF, the CT muscularity indices provide an opportunity to improve carcass conformation, a trait included in current breeding objectives.

Differences in muscularity of the HL and LR, assessed by the CT muscularity indices, and

eating quality were investigated between sexes (ram *vs* ewe lambs), breeds (SBF *vs* TEX) and progeny of high- and low-muscularity sires (HM, LM). TEX lambs had 16% greater muscularity than SBF in both regions, whilst differences between sire groups were 4%. Ewe lambs had slightly higher values of muscularity for the HL than rams but no difference was found for the LR. Meat from SBF lambs was more tender, and had stronger lamb flavour and higher overall liking scores than TEX meat. Sex had a weak influence with ram lambs having a stronger abnormal flavour and lower overall liking in the LR only. No significant differences in meat eating quality were found between HM- and LM-sired lambs, suggesting that improved muscularity would not have unfavourable effects on sensory traits.

Genetic parameters for the CT muscularity indices, predictions of carcass muscle and fat weights and CT muscle density were estimated. The estimates of heritabilities of the CT muscularity indices showed they were at least moderately heritable (from 0.38 to 0.92) in both breeds. CT muscle density, measured in the LR, had a moderate to high heritability in both breeds, and strong negative genetic and phenotypic associations with IMF and carcass fat weight. Little association was found between muscularity indices and CT muscle density, implying that improved muscularity would not have a negative effect on CT muscle density. These results suggest, overall, that the effect of selection for improved muscularity of sheep is likely to be favourable for carcass quality and neutral with respect to meat eating quality.

Because of the strong phenotypic and genetic associations with IMF, CT muscle density may be a promising selection tool to counteract possible negative effects of decreasing fatness on IMF and therefore eating quality. The inclusion of CT muscle density as a selection criterion allowed more favourable genetic responses in IMF, without further unfavourable increases in carcass fat weight or detrimental effects on leanness. Because increased economic values for IMF led to different expected gains in IMF and other traits in the breeding goal, the definition of the specific values depends on the desired gains in all traits by the industry. Positive returns for the industry from using CT muscle density at the second stage of selection can be obtained for all economic values included in this simulation. The economic benefits were maximised when the proportions of ram lambs CT scanned were 0.15.

DECLARATION

I declare that this thesis is my own composition and that the research described in it is my own work, except where otherwise stated.

June 2007

I wish to state that:

The index selection programmes used in Chapter 7 were based on programs previously developed and made available to me by Dr Ron Lewis, Virginia Polytechnic and State University, Blacksburg, VA, USA (formerly of Scottish Agricultural College, Edinburgh).

The Mathcad bivariate normal distribution programme used in Chapter 7 to calculate selection intensity at the second stage of selection was written and made available to me by Dr Peter Amer, Abacus Biotech, New Zealand.

PUBLICATIONS

Refereed publications

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Navajas, E.A, Glasbey, C.A., McLean, K.A., Lambe, N.R., Bünger, L. and Simm, G. 2004. Comparison of manual and automatic segmentation of muscle regions in spiral computed tomography images of sheep. *Proceedings of the British Society of Animal Science* **2004**: 35.

Navajas, E.A, McLean, K.A., Glasbey, C.A., Lambe, N.R., Fisher, A.V., Bünger, L. and Simm, G. 2005. Assessing muscularity in lambs using spiral CT scanning. *International seminar "Breeding for high quality lamb carcasses"*, Hamar, Norway, pp. 34-37.

Navajas, E.A., Charteris, A.J.L, McLean, K.A, Lambe, N.R, Fisher, A.V., Bünger, L. and Simm, G. 2005. Association among objective *in vivo* and post-slaughter assessments of muscularity in lambs. *Proceedings of the British Society of Animal Science* **2005**: 44.

Navajas, E.A., Lambe, N.R., Bünger, L., Glasbey, C.A., Fisher, A.V., Wood J.D. and Simm, G. 2006. Genetics of carcass shape and eating quality in sheep. In *New Developments in Sheepmeat Quality*, 9th Annual Langford Food Industry Conference, pp 59-64. University of Bristol.

Navajas, E.A., Lambe, N.R., Sawalha, R.M., Bünger, L. and Simm, G. 2006. Genetic parameters of *in vivo* muscularity in two divergent sheep breeds: preliminary results. *Proceedings of the 8th World Congress of Genetics Applied to Livestock Production*, CD-ROM communication n° 13-13. [Based on Chapter 6]

Book chapter

Navajas, E.A. and Simm, G., 2004. Animal breeding and genetics: DNA markers and marker-assisted selection. In *Encyclopedia of Meat Sciences* (eds. Jensen, W.K., Devine, C. and Dikeman, D), pp. 19-27. Elsevier: Oxford, Volume 1. [Appendix 1]

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1.1 Lamb markets and supply in the UK

Lamb consumption in the UK has declined over the last few decades. Levels of consumption have decreased from 160 g/person/week in the 1960's to current values of 50 g/person/week (Figure 1.1). One of the reasons for this reduction has been consumer preference for leaner meat than that produced from lamb carcasses (recently reviewed by QMS/MLC, unpublished). This is partly due to the consequences of excess fat consumption on human health (Higgs, 2000). Lamb fat is also less palatable that other animal fats due to a 'sticky mouth feel', which can be explained by its high melting point (Wood *et al.*, 2004).

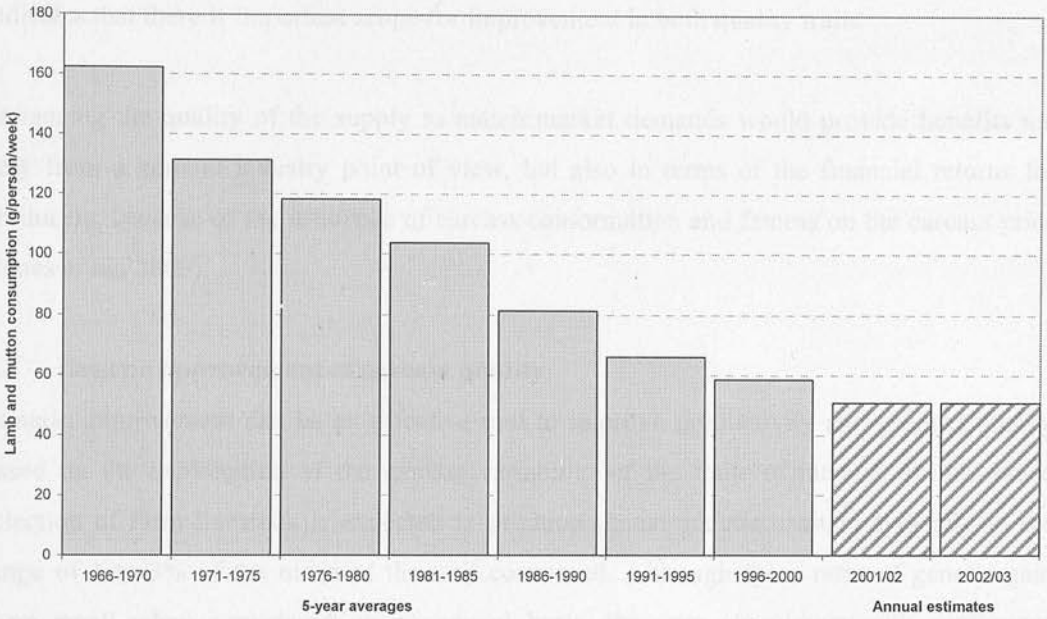


Figure 1.1: Lamb consumption in the UK. Annual estimates provided by the Expenditure and Food Survey (data from the UK), which replaced the National Food Survey (produced for Great Britain) in April 2001. Source: ONS (2006)

Consumer demand for leaner meat is also expressed in terms of carcass quality specifications. Market requirements in the UK are commonly defined based on the MLC carcass classification scores for fatness and conformation, which are widely used by the industry as a measure of carcass quality (Jones *et al.*, 2003). Carcass fatness is scored visually by assessing the amount of external fat. There are seven levels: 1, 2, 3L, 3H, 4L, 4H and 5, where 1 has least and 5 the most fat. Carcass conformation is determined by a visual appraisal of shape, taking into account the blockiness and fullness of the leg (MLC, 2002). It

is defined as the thickness of muscle and fat relative to the dimension of the skeleton (De Boer *et al.*, 1974). The possible scores are E, U, R, O and P where E indicates the best and P the worst conformation (MLC, 2002). Target carcass specifications for fatness for the main UK domestic and export markets are fat classes 2 or 3L. In terms of conformation, most markets prefer scores R or higher (Jones *et al.*, 2003).

On average over the last 4 years, only 54% of the UK lambs produce carcasses of the desired specifications (MLC, 2003, 2004, 2005, 2006, unpublished), which compromises the competitiveness of the UK sheep industry. The large proportion of carcasses that do not achieve the target specifications, because they are over-fat or have poor conformation, indicates that there is important scope for improvement in both quality traits.

Enhancing the quality of the supply to match market demands would provide benefits not only from a general industry point of view, but also in terms of the financial returns for producers, because of the influence of carcass conformation and fatness on the carcass price (Jones *et al.*, 2003).

1.2 Genetic improvement of carcass quality

Genetic improvement can be an effective tool to improve productivity and product quality based on the exploitation of the genetic variability of the traits of interest. Within-breed selection of farm livestock is expected to produce annual genetic changes typically in the range of 1 to 3% of the mean of the trait concerned. Although these rates of genetic gain seem small when considered on an annual basis, they are cumulative with continuous selection (Simm *et al.*, 2005).

Because of the economic relevance of carcass quality, the genetic improvement of these traits has been addressed in the UK. However, different carcass quality traits are included in the breeding goals of different breeds. In the case of UK hill sheep breeds, such as Scottish Blackface, the breeding goal traits related to carcass quality are carcass fat and conformation scores (Conington *et al.*, 2001). On the other hand, the selection index used by the main specialised meat breeds (terminal sire breeds), have the aim of increasing the weight of carcass lean while minimising any increase in the weight of fat at a given age (Simm and Dingwall, 1989).

Estimated genetic parameters for carcass traits indicate that they are under moderate to high

genetic control, which implies that there is scope to improve these traits by selection. Moderate heritabilities for lean and fat weights and proportions have been estimated in sheep. Estimates range from 0.30 to 0.40 for lean and fat proportions, respectively (Wolf *et al.*, 1981; Jones *et al.*, 1999). The average estimates reported by Simm (1992) for lean and fat weights were 0.27 and 0.29, respectively, whilst Van Heelsum *et al.* (2006) estimated a higher heritability of 0.46 for lean weight.

Heritabilities for visually appraised fat scores are moderate to low (0.17 - 0.33, Wolf *et al.*, 1981; Jones *et al.*, 1999; Pollott *et al.*, 1994; Conington *et al.*, 2001; Karamichou *et al.*, 2006b; Karamichou *et al.*, 2007), as are those for conformation scores (0.14 - 0.26; Wolf *et al.*, 1981; Jones *et al.*, 1999; Pollott *et al.*, 1994; Van Heelsum *et al.*, 2001; Van Heelsum *et al.*, 2006; Karamichou *et al.*, 2007). Extreme values of 0.09 and 0.52 were reported by Conington *et al.* (2001) and Karamichou *et al.* (2006b), respectively, in Scottish Blackface lambs.

Summarising, it seems obvious that the genetic variability of carcass quality traits in general is not a limitation for their improvement by breeding. However, the inclusion of these characteristics in breeding programmes has received less emphasis than other traits in the past because of the difficulties of measuring carcass quality traits on the selection candidates. The inclusion of such traits into breeding programmes requires either direct measurements on relatives of selection candidates or *in vivo* measurements on the candidates themselves and their relatives (Simm, 1992).

Selection in the UK has relied on indirect measurements taken on live animals. Breeding programs in terminal sire and hill breeds use ultrasound scanning, as it is a technique that can be readily used on a large number of animals directly on the farm. Ultrasound measurements of subcutaneous fat depth and muscle depth at the third lumbar vertebra are used as the selection criteria traits (Conington *et al.*, 2001; Simm *et al.*, 2002). A recent review of genetic parameters for economically important traits in sheep by Safari *et al.* (2005) shows that both ultrasound traits are heritable, with average heritabilities of 0.24 and 0.26 for muscle and fat depth, respectively.

There is evidence of successful changes of lamb carcass composition by selection. Genetic responses in lean composition have been achieved in purebred animals of the terminal sire breeds, with minimum changes in carcass fat, on a national scale in the UK (MLC, 2002). In

addition, Simm and Murphy (1996) reported that commercial lambs sired by rams selected for high lean growth index score had a greater weight of lean, lower estimated fat proportion and a higher saleable meat yield than lambs sired by unselected rams at the same carcass weight. However, the commercial lambs also had poorer conformation, which could be associated with the reduction of fat content in the carcasses.

Conington *et al.* (2006a) found that although selection in Scottish Blackface lambs based on multi-trait indices resulted in the improvement of the total index, the trends in breeding values for carcass fat and conformation scores were small and not significant. The authors discussed this as a potential consequence of the very low heritabilities of these traits previously reported by Conington *et al.* (2001), and the strong emphasis (high economic values) given to maternal traits.

1.2.1 Conformation and muscularity

One of the shortcomings of the existing carcass classification system is that conformation tends to be confounded with fatness (Kempster *et al.*, 1982; Jones *et al.*, 1999). This association between the traits is also manifested in terms of a positive genetic correlation between them (0.11, Conington *et al.*, 2001; 0.37, Jones *et al.*, 1999; 0.59, Pollott *et al.*, 1994), which limits the genetic improvement of conformation without increasing fatness.

Because the improvement of carcass shape using the conformation scores may lead to an undesirable increase in fat, measures of muscularity have received increasing attention as a measure of carcass shape that is independent of fatness. In addition to the enhancement of conformation, the improvement of muscularity may also be important because the shape of the cut has an effect on its attractiveness to consumers, who tend to prefer plump leg joints and large round chops (Chambers and Bowers, 1993; Laville *et al.*, 2004; Kukowski *et al.*, 2005).

Muscularity is defined as the shape of the muscle relative to the size of the skeleton (De Boer *et al.*, 1974). Although this definition may provide a way to objectively quantify muscularity, a comprehensive measurement of muscle depth is difficult to achieve. This limitation was overcome by the approach of Purchas *et al.* (1991) in which the weight of the muscle and the length of the bone are combined in a simple index that is practicable and consistent with the definition of muscularity. Although this approach has the advantage of providing a more robust muscularity index, it could initially not be used in live animals,

which restricted its utility in genetically improving muscularity. However, *in vivo* muscularity measures have been developed more recently using X-ray computed tomography (Jones *et al.*, 2002; Jones *et al.*, 2004) (more detailed information on these measurements of muscularity is given below).

Utilisation of the muscularity index would be of benefit in those breeding programmes in which the breeding objectives include carcass conformation and fat scores, as an indirect way of improving conformation. This may also provide the possibility of counteracting the slightly deleterious impact on carcass conformation when selecting to improve lean content (Simm and Murphy, 1996; Conington *et al.*, 2001; Karamichou *et al.*, 2007). Also, it has been pointed out by Jones *et al.* (2003) that the inclusion of muscularity into the lean growth index may improve the uptake of this tool by the industry.

1.3 Genetic improvement of meat quality

The lamb meat industry is under increasing pressure to improve several meat quality traits that are relevant from the consumer's point of view. The improvement of quality in general has been more difficult to address because quality can be defined in many ways and differs between countries (Sañudo *et al.*, 1998a; Hocquette and Gigli, 2005). In addition to the amount of fat, many other characteristics determine meat quality, including food safety (freedom from microbiological hazards), nutritional value (given by the chemical composition of the product) and sensory quality. Sensory quality is given by meat and fat colour (appearance), and tenderness, flavour and juiciness (palatability or eating quality) (Wood *et al.*, 1999; Hocquette and Gigli, 2005).

In addition to the difficulties of defining overall quality, the complexity and high cost of measuring meat quality traits, specially eating quality, have constrained the availability of selection traits, their genetic parameters and therefore the inclusion of quality traits in breeding programmes. Those estimates of genetic parameters that are available for meat quality traits and eating quality in beef and pigs show that they are heritable (reviews by de Vries *et al*, 2000; Burrow *et al.*, 2001; Lambe and Simm, 2004).

Because it is impossible to measure meat quality traits directly in the live animal, intramuscular fat (IMF) has been used in beef cattle and pork as a predictor of meat quality (Lambe and Simm, 2004). Breeding values for IMF measured by ultrasound are available in different countries including the USA and Australia (Hassen *et al.*, 2003). There appear to be

no studies on the utilisation of ultrasound scanning in sheep to assess IMF.

IMF is a meat trait of interest itself because it is associated with the nutritional value of meat. It is also relevant due to its positive association with meat eating quality. Higher contents of IMF are linked with higher tenderness, flavour and juiciness, and therefore it has a positive general effect on palatability (Savell and Cross, 1988). Although IMF plays a key role in terms of eating quality, the magnitude of the association has been controversial. Contents of 2 to 3% of IMF were identified by Savell and Cross (1988) as the minimum levels to achieve acceptable consumer satisfaction for grilled red meat cuts.

There is a perceived antagonism between selection for reduced fatness and maintaining or improving the eating quality of lamb (Simm, 1992). For example, recent findings showed that selection for leanness in Scottish Blackface was associated with darker meat colour and lower juiciness, as well as significantly lower content of IMF (Karamichou *et al.*, 2006b). However, it would be possible to limit reductions in IMF, whilst reducing other carcass fat, by employing some of the more advanced techniques, such as computed tomography for *in vivo* assessment of IMF (Simm, 1992).

Concerns regarding the effect of improving muscle mass in the carcass have been also raised, although the research evidence is not conclusive. Only two studies by Hopkins *et al.* (2005) and Johnson *et al.* (2005b) were found in which muscularity and/or muscle mass were considered as polygenic traits. Hopkins *et al.* (2005) reported an unfavourable effect of high estimated breeding values for muscling of Poll Dorset sires on the meat eating quality of their progeny. The phenotypic correlations between leg muscularity and meat quality traits estimated by Johnson *et al.* (2005b) were low and inconclusive. Unfavourable associations of lean meat yield and muscle growth with meat quality, in particular with tenderness, were reported in the Poll Dorset breed due to the Callipyge gene. This gene causes more rapid muscle accretion, and compact and leaner carcasses (Freking *et al.*, 1999). However, only mild effects of the Carwell gene (known to cause muscle hypertrophy in the *m. longissimus*) on tenderness were reported by Jopson *et al.* (2001), whilst Johnson *et al.* (2005a) found no effects of a QTL for increased muscling on meat quality traits in Texel lambs.

1.3.1 Molecular genetics and meat quality

The utilisation of molecular techniques to identify animals with the better genotypes to match market demands will allow much greater exploitation of major genes or other genes

with smaller but relevant effects on meat quality (Lambe and Simm, 2004). Gene- and marker-assisted selection schemes have been suggested as promising strategies for traits difficult to measure such as meat quality, in which a significant increase in accuracy of selection and selection response can be obtained by the inclusion of molecular marker data into breeding value estimation (Meuwissen and Goddard, 1996; Goddard and Hayes, 2002).

The majority of QTL detected in meat sheep so far, affect carcass composition and yield, rather than providing tools for direct enhancement of meat eating quality, though some have effects on quality, as pointed out by Navajas and Simm (2004) in a review of DNA markers and marker-assisted selection (see Appendix 1). More recently, a NZ joint venture consortium, Ovita®, has begun to market DNA marker tests, now via Catapult Genetics, identifying rams carrying one or two copies of the Carwell gene (LoinMAX®) (Simm *et al.*, 2006). A second test for a QTL segregating in Texels in NZ, associated with lower carcass fat and increased muscling (Johnson *et al.*, 2005a,b), is also commercially available (Simm *et al.*, 2006). These tests and another QTL for increasing muscle growth in Texel sheep in the UK (Walling *et al.*, 2004) are being validated in the UK (L. Bünger, personal communication).

There are significant programmes on QTL detection for meat quality traits in lamb in Australia and New Zealand (Campbell and Waldron, 2006). In the UK, information on QTLs affecting meat eating quality traits in the Scottish Blackface breed was recently reported by Karamichou *et al.* (2006c). They found evidences of QTL for a range of meat quality and carcass traits, including carcass and live weight, flavour of meat and meat colour. QTL were also reported in Scottish Blackface lambs for fatty acid composition of the *m. longissimus* (Karamichou *et al.*, 2006a).

1.4 Carcass and meat quality traits assessed *in vivo* by X-ray computed tomography

X-ray computed tomography (CT) scanning is a method for non-invasive imaging that was initially developed for use in human medicine. It allows different images in which the different tissues, organs and anatomical structures can be identified clearly. This technique provides valuable information to assess body composition objectively, as well as other novel traits, such as muscularity or IMF.

1.4.1 X-ray computed tomography

CT uses X-rays to generate cross-sectional, two-dimensional (2D) images of the body.

Images are acquired by rapid rotation of the X-ray tube 360° around the body of the subject. The radiation that is transmitted through the body is then measured by a ring of sensitive radiation detectors located on the gantry around the body (Jackson and Thomas, 2004).

The amount of radiation depends on the rate of attenuation of X-rays, which differs among tissues. The CT image is the result of a matrix of attenuation values (CT numbers), which are measures of density. They are expressed in Hounsfield units (HU) on a scale where water is zero and air is -1024 HU. Tissues less dense than water have CT numbers below zero. This is the case for fat, which has CT numbers in the range of -10 to -200, depending on its chemical composition and water content of the fat tissue. Muscle is more dense than water and therefore has positive CT numbers, usually in the range of 20 to 90. Bone has higher CT numbers, which approach 1000 when bone is very dense (Thompson and Kinghorn, 1992).

CT numbers are translated into grey level values to create a visual image of the cross-sectional area on a bitmap image produced on a PC screen (Wegener, 1993). Fat is shown as dark grey, muscle light grey and bone is white, as illustrated in the CT cross-sectional image in Figure 1.2.

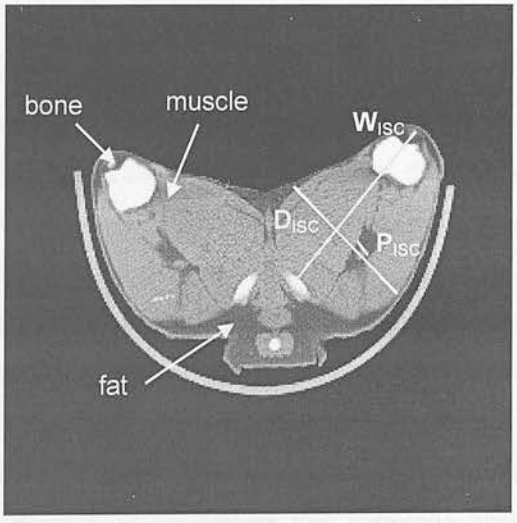


Figure 1.2: Cross-sectional CT image at the ischium (ISC), in which muscle (light grey), fat (dark grey) and bone (white) are indicated. The linear measurements used for the calculation of hind leg shape are illustrated. It is calculated as the ratio of the depth (D_{ISC}) of the hind leg muscle, minus the thickness of the popliteal fat depot (P_{ISC}), and the muscle width (W_{ISC})

1.4.2 Prediction of body components using CT cross-sectional images

CT provides very accurate estimation of sheep body composition (Young *et al.*, 2001a,b; Macfarlane *et al.*, 2006) based on the information provided by cross-sectional images of live animals. Suitable software procedures to extract and quantify this information have been developed using mathematical algorithms for image analysis (Glasbey and Robinson, 2002; Glasbey and Young, 2002).

Tissue information from CT images has been used to estimate body composition using two different approaches. The first approach is based on cross-sectional images take at specific anatomical positions (reference scans). Data on tissue areas from these images are then combined in prediction equations to predict the weights of lean, fat and bone in carcasses. This approach requires calibration trials in which animals are slaughtered and dissected into the main tissues after being CT scanned to produce the prediction equations that are applied to other animals of similar breed, sex and live weight. Genetic improvement of carcass composition may require modification of the prediction equations because of changes of quantity, and possibly distribution, of the different tissues in the body. Consequently, the accuracy of the estimation of body composition and the composition of the different regions based on prediction equations derived earlier may decrease over time.

The second approach is the Cavalieri method in which 15 to 20 cross-sectional images are obtained, evenly spaced along the long axis of the carcass, with the first position selected at random. The volume of the main tissues is calculated as the total area of each tissue from cross-sectional scans multiplied by the inter-scan distance (Roberts *et al.*, 1993; Young *et al.*, 1996; Brenoe and Kolstad, 2000). The advantage of this method is that it provides a direct measurement of the volumes of body tissues that is independent of the shape of the body (Szabo *et al.*, 1999). Tissue weights are then calculated by multiplying their volume and their mass densities, which are obtained from the average CT densities of each tissue based on the relationship between HU and density (Fullerton, 1980). Although the Cavalieri method produces no bias, it is more time consuming and expensive than the method based on reference scans.

Both approaches provide very accurate predictions of tissue weights. Macfarlane *et al.* (2006) reported accuracies of 92 to 97%, 98 to 99% and 86 to 93% for muscle, fat and bone, respectively. The R^2 values for the prediction of total carcass muscle from dissection based on the Cavalieri scans ranged from 95% to 97% for terminal sire breeds in the UK (Young *et*

al., 2001a). High values were also reported for total fat and bone by Young *et al.* (2001a). In this study, references scans and Cavalieri scans had a similar level of accuracy. Because the manual segmentation of the multiple cross-sectional images used in the Cavalieri approach is more time consuming and costly, Young *et al.* (2001a) concluded that it was not cost effective to use this approach. The reference scan approach is the main method used to provide *in vivo* predictions of carcass composition in sheep breeding programs using CT in the UK and New Zealand (Simm *et al.*, 2001; Nicoll *et al.*, 2002).

The development of automatic procedures to segment the larger number of CT images per animal used in the Cavalieri approach may make it possible to reduce costs and labour and improve cost-effectiveness. However, the random selection of the images along the body in each animal, which is inherent to this approach, makes this task much more difficult.

1.4.3 Assessments of muscularity in live animals using CT

Objective *in vivo* measurements of muscularity obtained from 2D CT images were investigated by Jones *et al.* (2002). A muscularity index for the carcass was defined based on the approach proposed by Purchas *et al.* (1991). This index combines the total carcass muscle predicted from CT using the reference scan approach and the length of the spine, which was assessed in a longitudinal 2D image called a topogram (Figure 1.3).

Two additional measurements of muscularity, for the lumbar region and the hind leg, were developed based on the combination of linear dimensions of muscle and skeletal structures (Jones *et al.*, 2002). These measurements are taken on the reference scans used for the prediction of carcass information that is utilised in breeding programmes in the UK. The hind leg shape was defined based on measurements taken on the reference scan at the ischium. This measurement of muscle shape is the ratio of the depth (D_{ISC}) of the hind leg muscle, minus the thickness of the popliteal fat depot (P_{ISC}), and the muscle width (W_{ISC}) (Figure 1.2). This ratio is multiplied by 10 and then averaged over both legs (Jones *et al.* 2002).

The measurement of muscularity for the lumbar region is given by the ratio between the depth of the *m. longissimus* (B_{CT}) measured on the reference scan at the 5th lumbar vertebra (Figure 1.4), and the spine length (Figure 1.3) (Jones *et al.*, 2002).

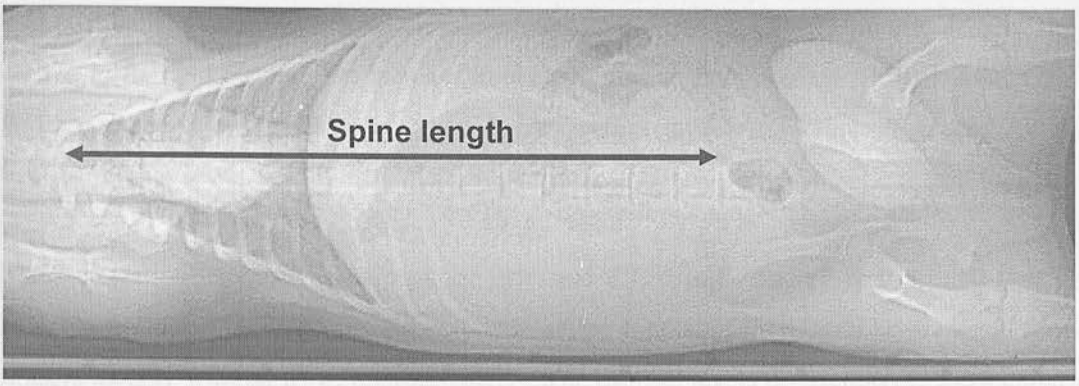


Figure 1.3: Topogram of a lamb in which the length of the spine is shown

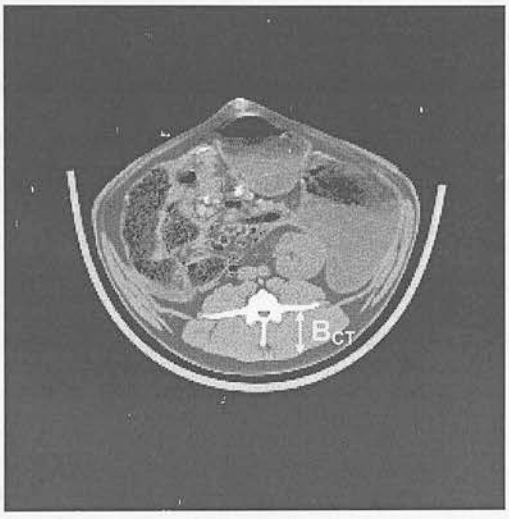


Figure 1.4: Cross-sectional CT image at the 5th lumbar vertebra. B_{CT} is the depth of the *m. longissimus*, which is combined with the spine length to assess muscularity in the lumbar region

The accuracy of these *in vivo* measures of muscularity was investigated by Jones *et al.* (2002). The phenotypic correlations between the CT and carcass measurements were, in general, smaller than 0.60.

1.4.4 CT muscle density as predictor of intramuscular fat

The CT number of each element of a CT image (pixel) represents the average amount of radiation absorbed by the tissue in that pixel given by the attenuation values (Wegener, 1993), which in turn is a function of tissue density and chemical composition (Goodpaster *et al.*, 2000).

This property gives CT the potential to describe the composition of tissues quantitatively.

The attenuation values of skeletal muscle *in vivo* determined by CT (CT muscle density) are related to its lipid content. An increase in skeletal muscle lipid content (IMF) is associated with a decrease in the CT attenuation values within muscle (Goodpaster *et al.*, 2000), because fat has lower density than the muscle fraction.

The association between the content of IMF and CT muscle density was investigated in sheep by Young *et al.* (2001a), who reported that the content of IMF could be predicted from muscle density with an R^2 of approximately 50%. Similar levels of phenotypic associations were found by Karamichou *et al.* (2006b) in Scottish Blackface lambs.

1.4.5 Spiral CT scanning

Spiral CT scanning is a more recent imaging technology in which the X-ray tube rotates continuously in one direction whilst the table on which the object to scan is lying is mechanically moved through the X-ray beam. The transmitted radiation takes on the form of a helix or spiral (Jackson and Thomas, 2004). Thus, this technology captures very detailed information from a continuous volume of contiguous slices in any specific region, or the whole body, rather than by collecting individual cross-sectional images. The potential of this technology has not been explored in livestock species.

1.5 Use of CT in breeding programmes

The very accurate CT predictions of carcass muscle and fat weights in sheep (Young *et al.*, 2001a,b; Macfarlane *et al.*, 2006) allows the genetic improvement of these traits to be accelerated by up to 50% compared to the use of ultrasound scanning only (Simm and Dingwall, 1989; Jopson *et al.*, 1995, 1997). However, the utilisation of this technology has restrictions because, in general, CT scanning is an expensive technique because of the cost of the scanning itself, the labour required for image analysis and transport expenses (Young *et al.*, 2001a) as CT units are usually situated at fixed locations requiring transport of animals to and from the CT facilities. The cost effective use of CT scanning in the industry requires a two-stage selection strategy. This strategy implies an initial screening of all selection candidates using ultrasound scanning on farm, which is a less accurate but more practical and cheaper *in vivo* technology. Animals selected on the information recorded in the first stage are then CT scanned. The final selection of rams is then carried out after completing the second stage.

The optimisation of two-stage selection programmes requires the calculation of the optimum

proportion of animals to be CT scanned and the optimum proportion to be selected for breeding. Investigations on the cost-effective use of CT for the main terminal sire breeds of sheep in the UK have been reported by Young *et al.* (2001a) and Macfarlane (2006). Results reported by Macfarlane (2006) indicated that optimal economical returns could be obtained when the proportion of animals put forward for CT scanning are 25%, 25% and 15% in three breeding schemes representing different population sizes and breeding structures of the Charollais, Suffolk and Texel sire reference schemes, respectively. Macfarlane (2006) also pointed out that currently only between 5% and 8% of the animals that are ultrasound scanned are CT scanned, which implies that more lambs than at present need to be CT scanned to achieve the full benefit from CT scanning. Work is required to encourage breeders with high-ranking animals, based on ultrasound scanning, to send these animals for CT scanning in order to maximise the genetic gain in carcass quality in a cost-effective way.

The inclusion of other quality traits, such as CT based muscularity indices and CT muscle density, may provide new tools to genetically improve carcass and meat quality and increase the exploitation of the benefits of CT scanning (Jones *et al.*, 2003). Evaluating the impact of adding the new characteristics requires knowledge of their genetic basis and genetic associations with other traits of interest. The possibility of predicting IMF using CT also provides the opportunity of increasing carcass leanness while limiting the reduction of IMF, which could minimise the possible antagonism between reducing fatness and eating quality.

1.6 Conclusions

CT scanning provides the opportunity to obtain *in vivo* information on novel traits such as muscularity and meat quality, which can be used in sheep breeding programmes in addition to the current traits.

Spiral CT scanning is a very valuable resource because of the new possibilities it offers as imaging technique. SCTS provide the opportunity to measure the muscle mass in any body region of interest, which not only gives the lean content of the different carcass regions but also information for the calculation of muscularity indices, and the dimensions of skeletal structures. Nevertheless, the exploitation of the potential of SCTS requires the development of new image analysis tools, which should be automatic because the high number of images contained in each spiral makes this task time-consuming if performed manually. These image analysis procedures should provide the information require to compute *in vivo* muscularity indices based on the approach proposed by Purchas *et al.* (1991) for the hind leg and the

lumbar region, in order to increase the accuracy of the CT measurements, particularly for the case of the hind leg.

Due to the economic relevance of several carcass traits such as carcass conformation and fat scores and lean content, the association of the muscularity indices with these traits should be determined. Similarly, further research is required on the relationship between muscularity and meat quality traits.

CT muscle density provides an accurate *in vivo* assessment of IMF, which is linked to lamb meat quality. Its inclusion in breeding programmes gives the opportunity to respond to consumers demand for leaner product by reducing fatness but maintaining eating quality, by minimising the reduction of IMF. However, the genetic associations among the traits of interest need to be quantified, as do the economic implications of these changes in the current two-stage selection strategies.

The above issues were addressed in this thesis, which had the following objectives:

(i) developing comprehensive *in vivo* assessments of muscularity in lambs using SCTS;

(ii) examining the relationship of the new muscularity indices with lamb carcass and eating quality;

(iii) exploring the associations among CT assessments of lamb carcass composition, muscularity and muscle density, and

(iv) investigating the possibility of limiting the antagonism between selection for reduced fatness and maintaining eating quality by introducing a CT predictor of IMF as an additional selection criterion in the sheep breeding programmes using CT.

1.7 Outline of the thesis

This thesis was carried out in the context of a multi-institutional project led by the Scottish Agricultural College. Data analysed in Chapters 3 to 6 was collected in a two-year experiment that is described in Chapter 2. More specific description of each analysis are given in the Materials and Methods section of each chapter.

Chapter 3 describes image analysis procedures developed for the SCTS with the objective of quantifying *in vivo* the muscle volume of the hind leg and lumbar region of lambs. The

accuracy was also investigated by comparing the CT values with information obtained from dissected lamb carcasses.

This new image analysis method was then applied on 120 Scottish Blackface and 120 Texel lambs in total. This information was combined with CT measurements of spine and femur lengths in new CT muscularity indices, which are explained in Chapter 4. The method to assess femur length *in vivo* is also explained there. The accuracy of the new CT muscularity indices was examined and compared with previous *in vivo* assessment of muscularity. The phenotypic associations with other carcass quality traits such as conformation and fat scores, and muscle and fat content, were also investigated in Texel and Scottish Blackface lambs.

Chapter 5 describes the effects of sex (ram *vs* ewes lambs), breed (Scottish Blackface *vs* Texel,) and sire group (high- and low-muscularity sires) on the muscularity of the hind leg and lumbar region of the carcass. The influence of these factors on meat eating quality, evaluated by taste panel, was also examined, with emphasis on the effect of muscularity. These results are also discussed in this chapter.

Genetic parameters for the new CT muscularity indices, CT muscle density and CT prediction of carcass lean and muscle, were estimated for both Scottish Blackface and Texel breeds to evaluate their potential use in sheep breeding programmes. Results are presented in Chapter 6.

The antagonism between reducing carcass fatness and increasing content of IMF content was examined in Chapter 7, by evaluating the impact of including IMF as a new breeding objective trait, and by adding CT muscle density in the second stage of selection as a selection criteria trait.

Chapter 8 contains a general discussion of the most important results of the previous chapters.

Chapter 2: General description of the experimental work

2.1 Introduction

Information analysed in this thesis was recorded as part of a larger study carried out by the Scottish Agricultural College (SAC), in collaboration with Biomathematics and Statistics Scotland (BioSS), Silsoe Research Institute and the University of Bristol, with one of its aims being investigating *in vivo* measurements of muscularity and their associations with meat quality.

The objective of this chapter is to give a general overview of the experimental work, including its design and description of the main protocols.

2.2 Experimental flocks

A flock of approximately 250 mixed-age ewes comprising approximately half Scottish Blackface (SBF) and half Texel (TEX) was established at SAC. Ewes were artificially inseminated in 2002 and 2003 with semen from sires of their own breed. Sires were selected from among those that had X-ray Computer Tomography (CT) information available, and they were used in both years. To produce lambs within each breed with increased variation in muscularity, rams were selected that were divergent for muscularity in the hind leg. Muscularity was measured using the 2D approach proposed by Jones *et al.* (2002), referred here as hind leg muscle shape. Sires were defined as high muscularity (HM) or low muscularity (LM) according to these measurements taken at previous scanning events. Five HM and five LM sires were used per breed, with approximately equal numbers of ewes in each sire group within year. Following AI, ewes were run with back-up rams from the same sources as the AI rams. The raw means and standard deviations of hind leg shape within each of these groups for each breed are shown in Table 2.1.

Table 2.1: Means (standard deviations) of hind leg shape ratios of sire groups

Breed[†]	HM sires[‡]	LM sires	Ratio HM/LM
TEX	7.84 (0.36)	6.30 (0.27)	1.24
SBF	6.06 (0.26)	4.62 (0.15)	1.31

[†] TEX: Texel; SBF: Scottish Blackface
[‡] HM: high muscularity; LM: low muscularity

Four hundred and seventy three ewe and ram lambs (SBF n=232; TEX n=241) were slaughtered in this experiment in the years 2003 and 2004 (Table 2.2). SBF lambs were from 11 sires, with between 2 and 49 lambs per sire (average = 21) from an average of 16 dams

per sire. TEX lambs were from 10 sires, with between 14 and 37 lambs per sire (average = 24) from an average of 17 dams per sire. (Note that minor differences in the number of animals reported in the following chapters are explained by missing CT data or sire identification).

2.3 Finishing and slaughter criteria

Lambs were grazed on lowland paddocks in mixed-breed groups from birth to slaughter. In the period between weaning and slaughter lambs grazed on Ryegrass/Clover leys. Although lambs were finished off grass, they were provided with supplements from autumn onwards because of the lower grass availability and quality. In order to minimise feed effects on meat quality, only dried grass pellets (2003) and haylage or hay (2004) were given. Lambs were CT scanned on a maximum of four occasions during the growing period and pre-slaughter. Lambs were slaughtered in five batches in 2003 and six batches in 2004. Selection for each batch depended on live weight and condition score. Lambs were finished at a target condition score of 3 (on a subjective scale of 0 to 5) and a minimum live weight of 35 kg in 2003 and 32 kg in 2004, due to slower growth rates in the second year.

Table 2.2: Numbers of lambs slaughtered at finishing and one month later by year of slaughter and breed

Year of slaughter	Breed[†]	Time of slaughter		Total
		At finishing	One month later	
2003	TEX	62	60	123
	SBF	47	46	93
2004	TEX	58	59	117
	SBF	74	66	140
Total		241	232	473

[†] TEX: Texel; SBF: Scottish Blackface

Each finishing batch was of mixed breed and sex. Age at finishing ranged from 91 to 202 d, with an average of 139 d (TEX, 134 d; SBF, 144 d). Half of the lambs in each finishing batch (balanced for breed and sex) were slaughtered at finishing, when they were CT scanned. The other half of the lambs of each batch were slaughtered 30 days later, due to the withdrawal period for the sedative used as a pre-requisite for CT scanning, to allow taste panel analyses. Lambs in the second half of each batch continued to be managed to achieve

positive growth until slaughter, with a diet based on grass (and grass-based supplements when required), as described above. Average daily weight gains from weaning to slaughter were 56 g/d for TEX and 34 g/d for SBF, averaged over both years. Number of lambs slaughtered at finishing and one month later, by breed and year, are presented in Table 2.2. The distribution by sex and sire group within each breed is given in Table 2.3.

Table 2.3: Numbers of lambs slaughtered by breed, sire group (high and low muscularity), sex and year of slaughter

Breed† –year	High muscularity		Low muscularity		Total
	Male	Female	Male	Female	
TEX - 2003	23	40	31	29	123
TEX - 2004	33	33	21	30	117
TEX – total	56	73	52	59	240
SBF - 2003	18	28	34	13	93
SBF - 2004	40	24	36	40	140
SBF – total	58	52	70	53	233

† TEX: Texel; SBF: Scottish Blackface

2.4 CT scanning of lambs

The protocol used for CT scanning of lambs in each event included CT imaging technologies already being used such as reference scans and topograms (Figure 2.1), as well as spiral CT scans (SCTS) which were investigated in this study:

(i) Topogram: ventro-dorsal view is initially used to position the reference and spiral scans. Spine lengths were also measured on the topogram and combined with other CT measures in muscularity indexes for the lumbar region (Jones *et al.*, 2002; Chapter 4).

(ii) Reference scans: axial scans at fixed anatomical positions, ischium (caudal to the pelvis), 5th lumbar vertebra (LV5) and 8th thoracic vertebra (TV8). Reference scans were used to predict carcass composition based on prediction equations described by Young *et al.* (2001a) and Macfarlane *et al.* (2006).

(iii) SCTS: contiguous axial scans that allow the reconstruction of images in 3 dimensions to enable then to be viewed in different planes. Two contiguous spiral scans, from the upper third of the tibia to the second cervical vertebra, were collected. They provided data for new muscularity indices for the hind leg and

lumbar region (described in Chapters 3 and 4).

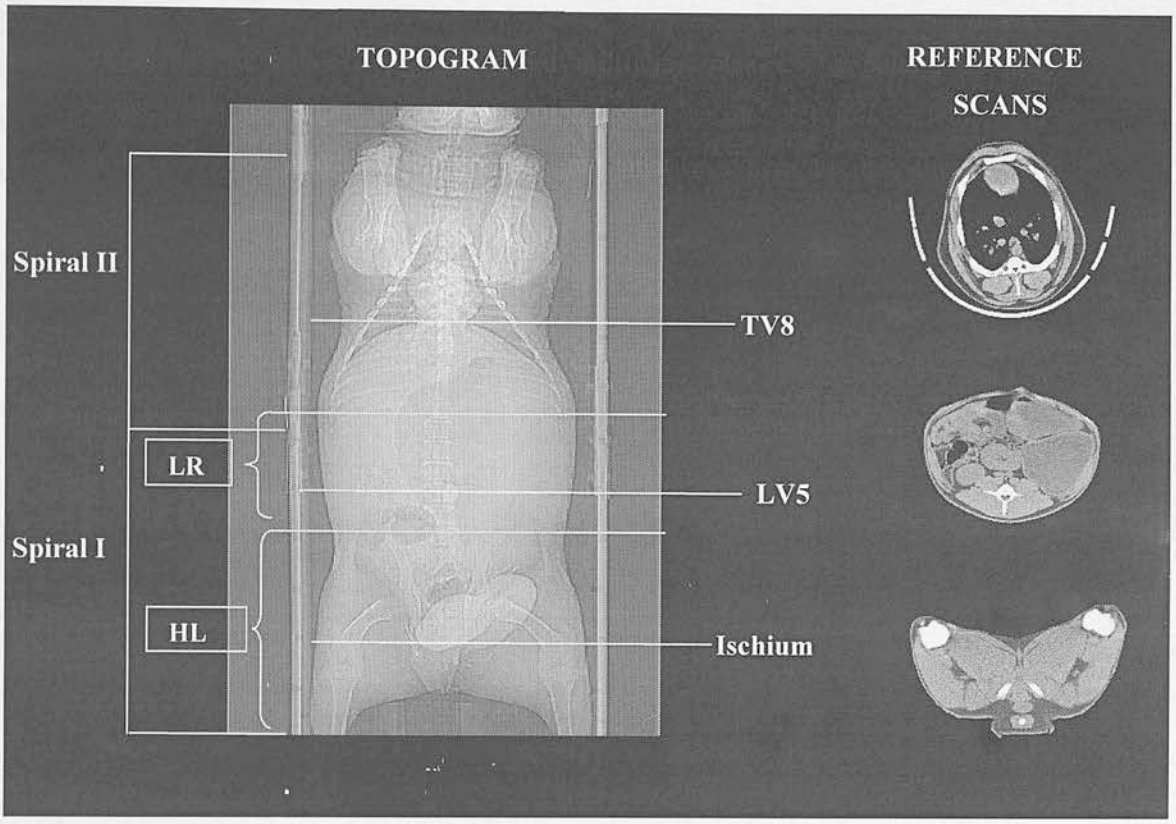


Figure 2.1: Topogram and references scans. Positions of reference scans (TV8, LV5 and Ischium) and spiral CT scans (Spirals I and II) are indicated, as well as the lumbar region (LR) and hind leg (HL)

2.5 Carcass and meat quality information

Quality of lamb carcasses was assessed in terms of size, shape and composition of the carcass. Hot and cold carcass weights were recorded and MLC carcass conformation and fat scores were also assessed (MLC, 2002). MLC conformation scores were transformed to numerical values were E=5, U=4, R=3, O=2 and P=1, and fat classes to their equivalent subcutaneous fat proportions, where fat class 1=4%, class 2=8%, class 3L=11%, class 3H=13%, class 4L=15%, class 4H=17% and class 5=20% (Kempster *et al.*, 1986). Averages and standard deviations for these traits, by year of slaughter and breed, are presented in Table 2.4. Conformation and fat score were also evaluated using the 15-point scales as proposed by De Boer *et al.* (1974) (Chapter 4).

Approximately 20% of the carcasses were fully dissected, as well as hind legs of all

carcasses, into the main tissues: subcutaneous fat, intermuscular fat, bone and muscle. Carcasses for full dissection were randomly sampled within batch/breed/sire group/sex. Equations to predict the total carcass composition and tissue composition of the other primals, from the hind leg dissections were estimated based on these data, as described in Appendix 2. The hind leg dissection was used to estimate carcass composition in the remaining 80% of the carcasses based on these equations.

Table 2.4: Carcass weights and conformation and fat scores: averages (standard deviations) by year and breed

Year	Trait	TEX†		SBF	
2003	Hot carcass wt	19.0	(2.7)	17.4	(1.8)
	Conformation	3.6	(0.6)	2.7	(0.5)
	Fat	5.5	(2.3)	10.0	(2.4)
2004	Hot carcass wt	17.7	(2.5)	14.2	(1.6)
	Conformation	3.5	(0.7)	2.0	(0.5)
	Fat	5.4	(2.2)	8.7	(2.8)

† TEX: Texel; SBF: Scottish Blackface

Meat quality traits analysed in this thesis were the content of intramuscular fat (IMF) and eating quality of main muscles of the lumbar region and hind leg.

The content of IMF in the *m. gracilis* (hind leg) and *m. longissimus lumborum* (lumbar region) were determined by chemical analysis. IMF in the hind leg was estimated as the total amount of all phospholipid and neutral lipid fatty acids. The day after slaughter, the *m. gracilis* was dissected from the left hind leg and half the muscle was used for fatty acid analysis. After thawing, any small amounts of adhering adipose or connective tissue were removed and the muscle was homogenized in a food processor. The fatty acids were extracted by direct saponification, methylated and analysed by gas-liquid chromatography, following the method of Doran *et al.* (2006).

In the case of the *m. longissimus lumborum*, IMF was extracted from the muscle samples, after being blended and dried, using petroleum ether (B.P. 40-60 °C) as the solvent in a modified Soxhlet extraction using an automatic Gerhardt Soxtherm 2000 unit (Gerhardt Gmbh, Koningswinter, DE).

Eating quality was evaluated by a trained taste panel at the University of Bristol on muscle samples from the *m. semimembranosus* (hind leg) and *m. longissimus lumborum* (lumbar region) of those lambs slaughtered one month after CT scanning. Muscle samples were removed from the right carcass side the day after slaughter, and vacuum packed. After an ageing period of 7 d, samples were frozen. For the sensory analysis, samples were thawed at 4 °C overnight, then cut into 2 cm thick steaks and cooked until the internal temperature reached 75 °C. Between 6 and 10 assessors rated 2 cm^3 samples of each muscle. The assessors used 8-point category scales (Sañudo *et al.*, 1998a), to evaluate the following traits: texture (1 – extremely tough, 8 – extremely tender); juiciness (1 – extremely dry, 8 – extremely juicy); lamb flavour intensity (1 – extremely weak, 8 – extremely strong), abnormal flavour intensity (1 – extremely weak, 8 – extremely strong) and overall liking (hedonic) (1 – dislike very much, 8 – like very much).

Chapter 3: *In vivo* measurements of muscle volume by automatic image analysis of spiral computed tomography scans

3.1 Introduction

Computed tomography (CT) is a non-invasive method that can be used to measure body composition accurately in live animals (Young *et al.*, 2001b). Organs, anatomical structures and main tissues can be visualised in the CT images, based on the X-ray attenuation differences among body tissues. CT scanning is being utilised in sheep breeding programmes in the UK, New Zealand and Norway to enhance genetic improvement of carcass composition using *in vivo* measurements of body composition (Simm *et al.*, 2001; Nicoll *et al.*, 2002; Kvame and Vangen, 2006).

Two main approaches are available currently to predict body composition from CT scans. The first is based on the utilisation of information from cross-sectional CT images at fixed anatomical positions in the animal (reference scans) to predict the proportion of muscle, fat and bone in the carcass (Young *et al.*, 2001b). This approach requires calibration trials in which animals are slaughtered after being CT scanned to produce the prediction equations that are applicable to animals of similar breed, sex and live weight. The second approach is the Cavalieri method in which 15 to 20 cross-sectional images are obtained, evenly spaced along the long axis of the carcass, with the first position selected at random. Body composition is calculated as the total area of each tissue across cross-sectional scans multiplied by the inter-scan distance (Roberts *et al.*, 1993; Young *et al.*, 1996; Brenoe and Kolstad, 2000). The advantage of this method is that it provides a direct measurement of the volumes of body tissues that is independent of the shape of the body (Szabo *et al.*, 1999). Although this method produces no bias, it is more time consuming and expensive than the method based on reference scans.

Spiral CT scanning is a new imaging technology in which multiple contiguous cross-sectional images of a known thickness are collected. This new technology captures detailed information on any specific body region, or the whole body, by acquiring data on the entire anatomical regions of interest rather than collecting individual cross-sectional images (Imaginis, 2005). The data from spiral CT scans (SCTS) can be computer-reconstructed in three dimensions (3D), giving the possibility of a comprehensive assessment of characteristics that are defined in terms of shape. For measurements of tissue volume and assessment of tissue shape in 3D re-constructions, image analysis procedures are needed to isolate the body components to be investigated. The development of automatic procedures for image analysis of SCTS is of high priority due to the large amount of information contained in these scans.

The aims of this study were: (i) to describe an automatic image analysis method developed for SCTS, with the objective of calculating the volume of muscle in the hind leg and lumbar region in lambs; and (ii) to evaluate the accuracy of this method by comparing the *in vivo* muscle volume to the muscle weight measured after dissection.

3.2 Materials and methods

Ewe and ram lambs of two breeds, Texel (TEX) and Scottish Blackface (SBF), were included in this study (numbers are given in Table 3.1). These lambs were from a larger study carried out by the Scottish Agricultural College (SAC), in collaboration with Biomathematics and Statistics Scotland (BioSS), Silsoe Research Institute and the University of Bristol, with the objective of investigating *in vivo* measurements of muscularity and their associations with meat quality.

Lambs were grazed in single sex, mixed-breed groups at a SAC experimental farm, from weaning until selected for slaughter according to the criteria commercially used in the UK, which is based on condition score and live weight. The target condition score for finishing was 3 (on a scale of 0 to 5). The thresholds for live weight were 35 and 32 kg in 2003 and 2004, respectively, due to slower growth rates in the second year.

3.2.1 CT scanning

Finished lambs were CT scanned before slaughter using a Siemens Somatom Esprit scanner at the SAC-BioSS CT unit in Edinburgh. Food was withheld for a minimum of four hours before scanning. Lambs were scanned lying on their backs with their hind legs extended and were gently restrained in a specially designed cradle. To minimise stress and movement during scanning, lambs were lightly sedated with Rompun™ (0.1-0.2 mg xylazine hydrochloride/kg body weight). Lambs were individually weighed immediately before scanning to ensure the right sedative dose.

The CT scanning protocol included two SCTS: the first from the proximal third of the tibia to the last rib and the second from the last rib to the 4^{th}-5^{th} cervical vertebra. Figure 3.1 illustrates the position of the SCTS, as well as the hind leg and lumbar region, in a ventro-dorsal CT scan (topogram) of a lamb. The information for this study was obtained mainly from the first SCTS (SCTS I; spiral I in Figure 3.1), which comprises the hind leg and lumbar region. Cross-sectional images of the second spiral (SCTS II; spiral II in Figure 3.1)

were also analysed in the few cases when the lumbar region was not completely included in SCTS I. The SCTS of each lamb for both regions included approximately 60 cross-sectional images.

3.2.2 Image analysis of the SCTS

The image analysis of SCTS has three steps: (1) segmentation to remove non-carcass portions of the images; (2) measurement of muscle areas in the segmented images, and (3) calculation of muscle volume. All the image processing algorithms described in this paper were implemented in an interactive computer program, Sheep Tomogram Analysis Routines software (STAR; Mann *et al.*, 2005), written in Fortran90 and VisualBasic.

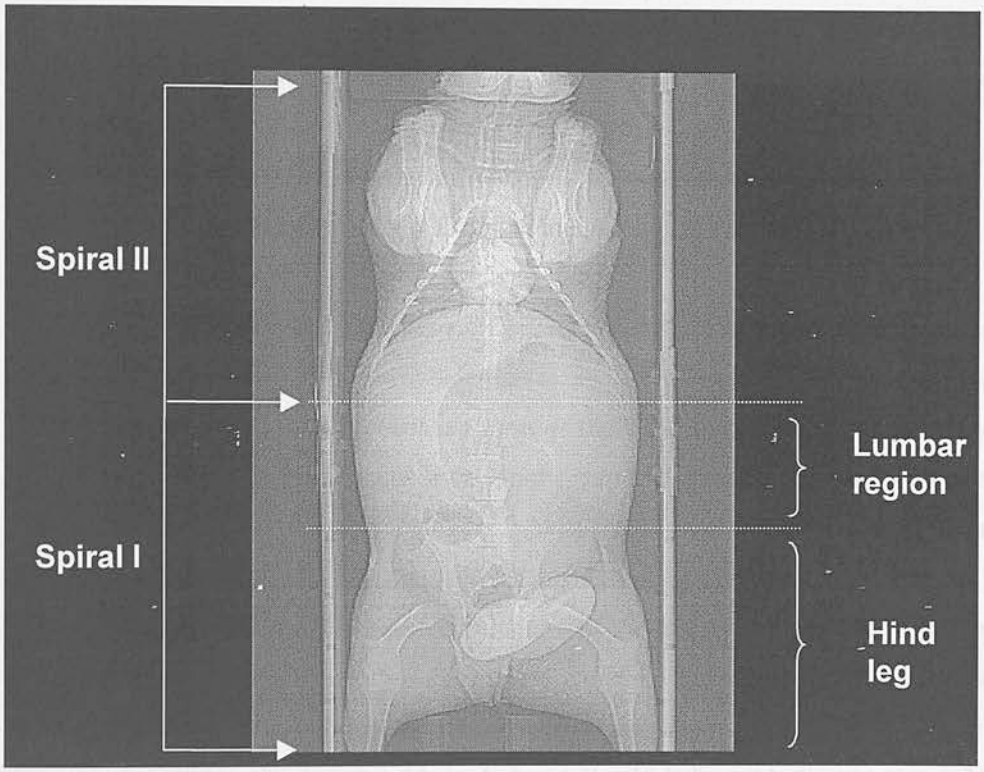


Figure 3.1: The regions of the body included in each spiral CT scan and those that correspond to the hind leg and lumbar region are indicated in a CT ventro-dorsal image of a lamb

3.2.2.1 Segmentation

SCTS from 10 SBF lambs, which were CT scanned and slaughtered in a pilot trial in 2002, were manually segmented by three experienced operators to provide the information required for the development of the new segmentation algorithm. In each cross-sectional image, a boundary (segmentation path) that excluded the non-carcass components was drawn and

only the area within the boundary was kept.

Due to the differences in muscle shape along the hind leg and between the hind leg and the lumbar region, different segmentation paths were applied in specific sub-regions. The program automatically identifies the skeletal landmarks that define these sub-regions, and allow these to be checked by the operator before proceeding with the segmentation. This interactive step also allows the manual identification of the landmarks in case the software fails to do so automatically (Glasbey *et al.*, 2004; Navajas *et al.*, 2004).

Four landmarks were defined for the hind leg due to the changes of shape in this region, whilst in the lumbar region the same segmentation path is applied throughout, because the shape is more homogeneous, and a single landmark specifies the end of this region. The five landmarks are shown for a representative lamb in Figure 3.2, together with a silhouette of its skeleton. Landmarks 2 to 5 can be recognised automatically from the skeleton, whereas landmark 1 (the first image in which there is no physical separation between right and left hind legs) is identified automatically from the individual CT images (see Figure 3.2).

Once the landmarks have been specified, the non-carcass components are removed automatically from the cross-sectional images by the program. A different segmentation algorithm is applied to images between each of the five landmarks, using modifications of the method proposed by Glasbey and Young (2002). The identification of one of the segmentation paths is illustrated in Figure 3.3. In this case, the segmentation path identifies the boundary of the muscle and removes the sexual organs from one of the images between landmarks 2 and 3. The program first identifies the path end-points, denoted by white squares in Figure 3.3(a), which are the highest points on the boundary of the animal on the two sides. Then a filter is applied to a window of pixels between these end-points. The filter returns a low value at a location if pixels immediately below this location are of a lighter grey shade (corresponding to muscle) and pixels immediately above are of darker shades (i.e. fat or air), and otherwise returns a higher value. If we denote by $Y_{i,j}$ the pixel with (descending) row index i, column index j, and by $Z_{i,j}$ the filter output, then

$$Z_{i,j} = \sum_{k=-5}^{0} \max\left(Y_{i+k,j} + 56,\ 0\right) + \sum_{k=1}^{5} \left|Y_{i+k,j} - 44\right|,$$

where -56 HU is chosen as a typical CT value for fat and +44 HU as a typical value for muscle, and k is the index for summing over the values of the 5 pixels below and above

location (i,j).

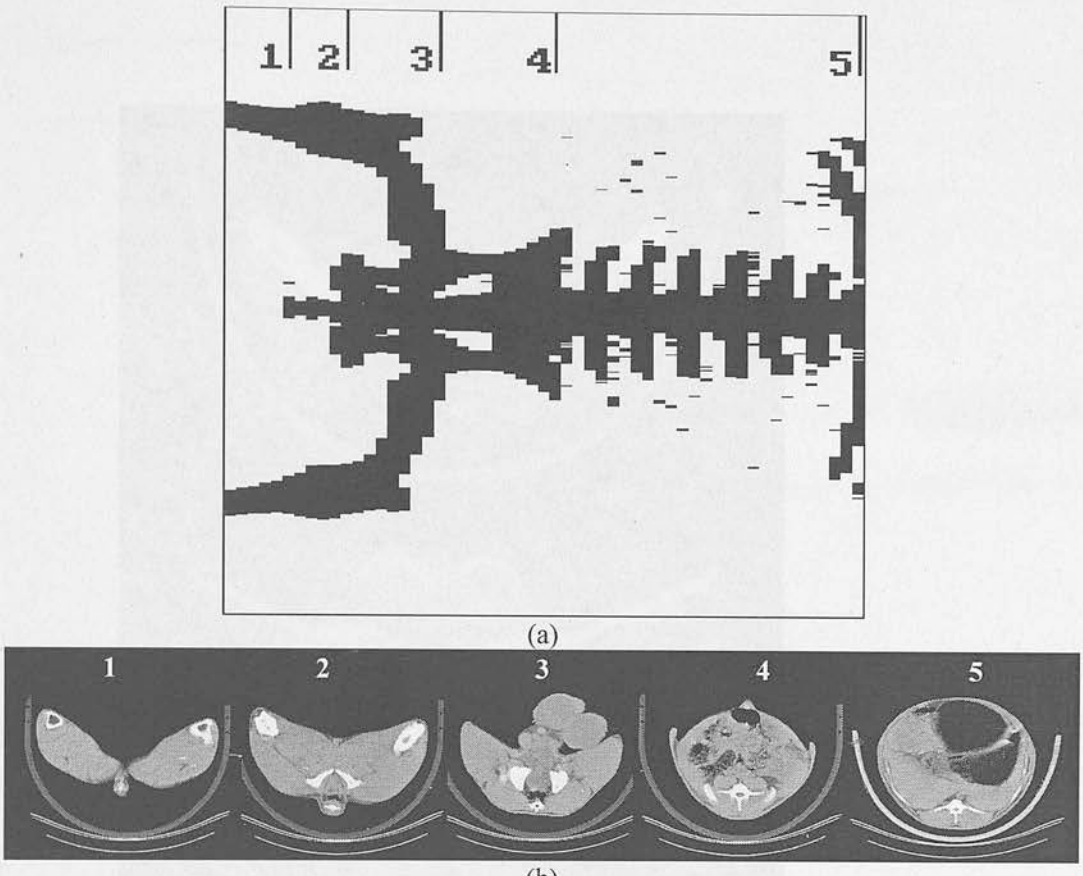


Figure 3.2: (a) Silhouette of skeleton of a lamb with five landmarks shown, and (b) examples of cross-sectional images of SCTS. Landmark 1 is the first image in which there is no physical separation between the right and left legs, landmark 2 is the first image in which the two sides of ischium bone join, landmark 3 is the first image in which the pelvis opens to the gut, landmark 4 is the last image including the pelvis; and landmark 5 is the first image in which a rib joins the backbone

This filter was chosen because the segmentation boundary that needs to be identified by the computer program has muscle and bone below, and fat and air above. The filter output is shown in Figure 3.3(b). Finally, the program finds the path that links the two end-points and incurs the minimum cumulative cost in filter values. The path is constrained to traverse the left half of the array in either horizontal steps or diagonally-downward steps, and the right half in either horizontal or diagonally-upward steps. The path, which is shown superimposed in Figure 3.3(c), can be found very quickly using dynamic programming, as explained by

Glasbey and Young (2002). Figure 3.4(b) shows this boundary superimposed on the CT image. Segmentation paths for other images are also illustrated in Figure 3.4.

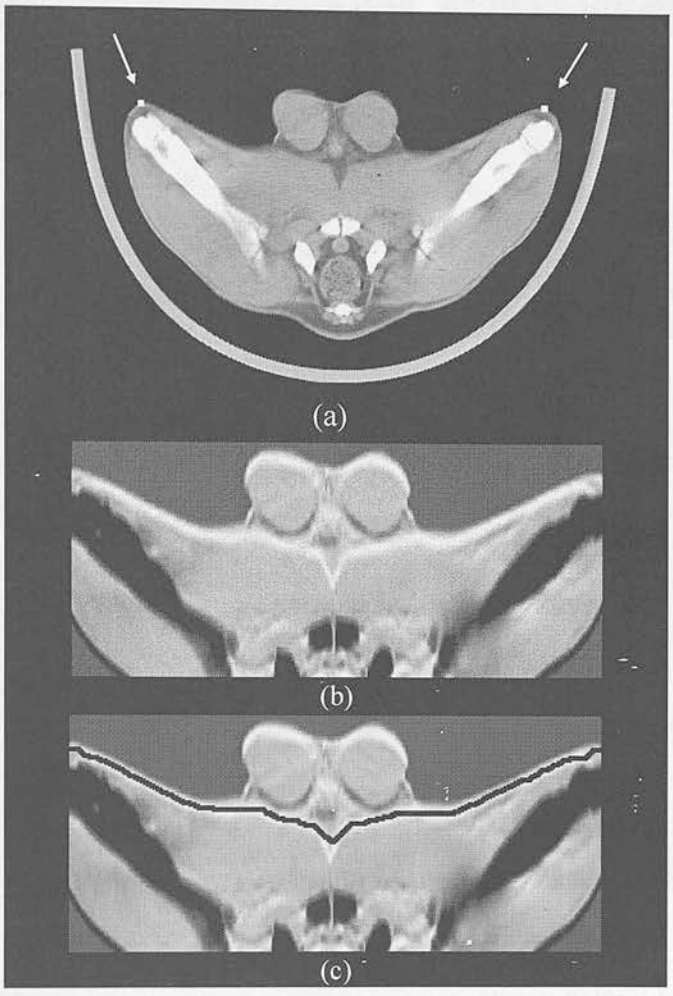


Figure 3.3: Demonstration of how one of the segmentation paths is found: (a) CT image, with end-points of path shown as white squares, (b) output of filter applied to window of pixels between the end-points, with lower values of the filter displayed as lighter shades of grey, (c) output of filter with optimal path between two sides shown as black line

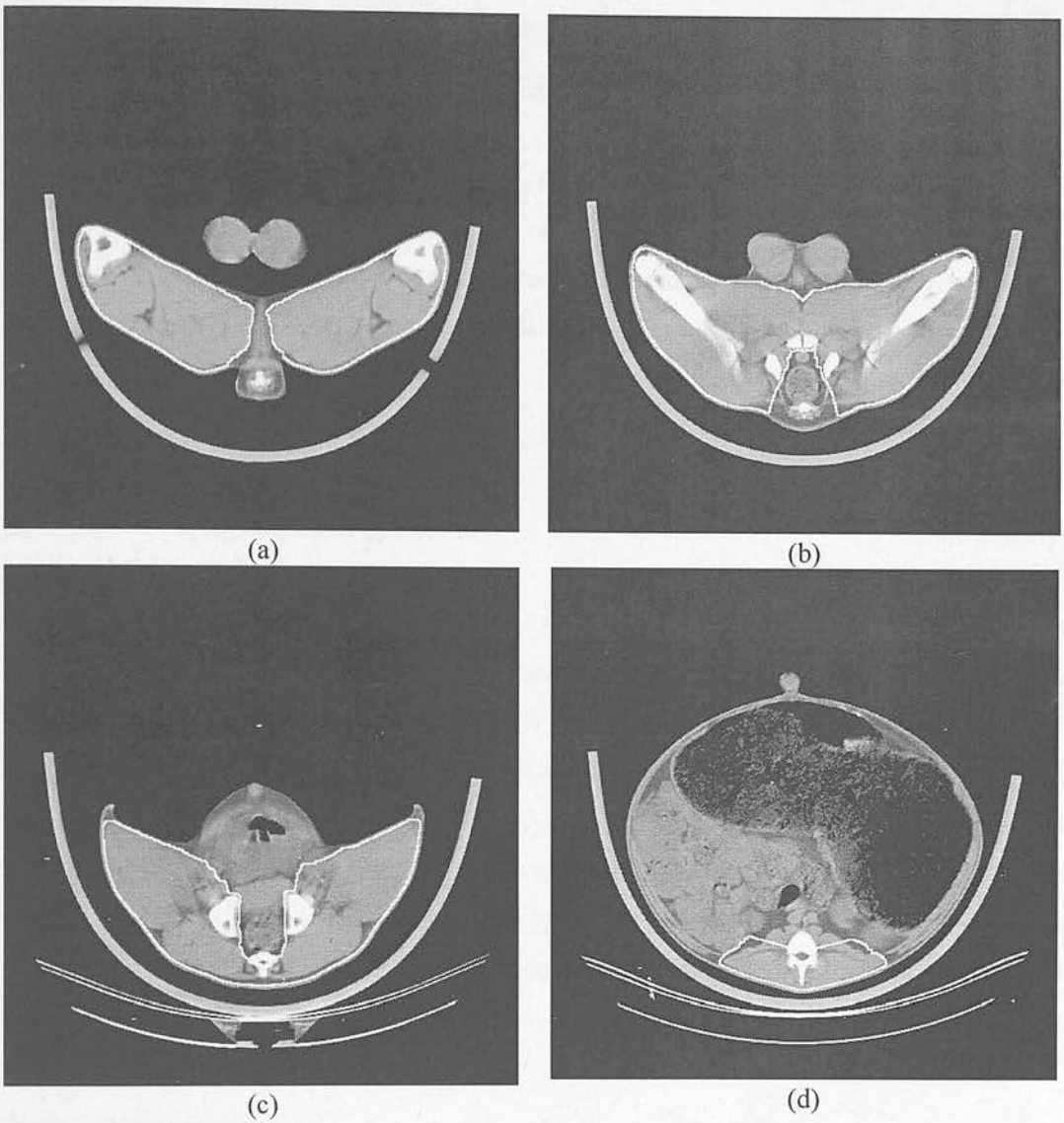


Figure 3.4: Four illustrative images from a representative lamb, with segmentation boundaries super-imposed (white lines): (a) removal of tail and connected tissues, for images between landmarks 1 and 2, (b) removal of sexual organs and tissues around anus, for images between landmarks 2 and 3, (c) removal of internal organs and muscles other than leg muscles, for images between landmarks 3 and 4, (d) isolation of *m. longissimus lumborum* and *m. multifidus*, for images between landmarks 4 and 5

3.2.2.2 Measurement of muscle areas

The second step is quantifying the muscle area in each segmented cross-section image of the SCTS. This is done simply by counting the number of pixels in the segmented region which have CT values in the range corresponding to muscle tissue (see, for example, Glasbey and Horgan, 1995, pp. 156-157). The range of -10 to +93 Hounsfield units was used in this

study. A more sophisticated approach is to take account of mixed pixels, as in Glasbey and Robinson (2002), though in practice the authors found that the differences in estimated areas were very small.

3.2.2.3 Calculation of muscle volume

The hind leg is defined by the 1st and 4th anatomical landmarks (see Figure 3.2), whilst the lumbar region comprised those cross-sectional images from the hind leg to the first image in which the ribs touch the spine (landmark 5). The volume of muscle in the hind leg ($HLMV_{CT}$) and the lumbar region ($LRMV_{CT}$) were calculated as the sum of the products of the total muscle area multiplied by the depth of the cross-sectional images (8 mm) across all cross-sectional images in each region.

3.2.3 Dissection data

Lambs were finished in five and six batches in 2003 and 2004, respectively, and transported to the University of Bristol, where they were slaughtered. The accuracy of assessment of muscle volume with the automatic image analysis method was investigated using SCTS and dissection data of the lambs slaughtered 4 days after CT scanning (no. = 240). The hind legs of all the lambs were dissected and muscle weights were measured ($HLMW_D$). Data on the total muscle weights of the lumbar region ($LRMW_D$) were available on 50 of these lambs, which were fully dissected. These lambs were sampled randomly within batch, breed and sex.

3.2.4 Data analysis

The association between the *in vivo* and post-mortem data was analysed using multiple regression analysis (Genstat, 2004). Breed, year of slaughter and sex were fitted as class effects to test the influence of these factors on this association. The general model was:

$y_{ijk} = a + B_i + Y_j + S_k + b\, x_{ijk} + e_{ijk}$

with y_{ijk} = $HLMW_D$ or $LRMW_D$; a = intercept; B_i = breed (i = TEX, SBF); Y_j = year of slaughter (j = 2003; 2004); S_k = sex (k = female; male); b = regression coefficient; x_{ijk} = $HLMV_{CT}$ or $LRMV_{CT}$; e_{ijk} = residual error. Two-way interactions between class effects were tested.

3.3 Results

Table 3.1 shows the raw means, standard deviations and coefficients of variation for dissected muscle weights and CT muscle volumes of TEX and SBF lambs for both body

regions. The averages values obtained for $HLMW_D$ and $LRMW_D$ seem to be higher in the TEX lambs than in the SBF by 47% and 27%, respectively. The relative differences between the breeds for the means of $HLMV_{CT}$ and $LRMV_{CT}$ were similar to the difference observed when comparing the dissection information.

Table 3.1: Means, standard deviations (s.d.) and coefficients of variation (CV) for dissected muscle weight ($HLMW_D$ and $LRMW_D$, g)† and CT muscle volume ($HLMV_{CT}$ and $LRMV_{CT}$, cm^3)† and in the hind leg and lumbar region of Texel and Scottish Blackface lambs

HIND	Texel		Scottish Blackface		Total	
LEG	$HLMW_D$	$HLMV_{CT}$	$HLMW_D$	$HLMV_{CT}$	$HLMW_D$	$HLMV_{CT}$
No. animals	120		120		240	
Mean	1965	1935	1341	1328	1652	1631
Maximum	2688	2740	1737	1808	2688	2740
Minimum	1478	1338	979	955	979	955
s.d.	251.0	260.4	178.1	197.2	380.5	381.8
CV (%)	12.8	13.5	13.3	14.9	23.0	23.4
LUMBAR	Texel		Scottish Blackface		Total	
REGION	$LRMW_D$	$LRMV_{CT}$	$LRMW_D$	$LRMV_{CT}$	$LRMW_D$	$LRMV_{CT}$
No. animals	26		24		50	
Mean	464	348	366	277	417	314
Maximum	623	487	511	388	623	487
Minimum	317	251	272	177	272	177
s.d.	68.2	65.9	57.3	54.0	79.8	69.6
CV (%)	14.7	19.0	15.7	19.5	19.2	22.2

† $HLMW_D$ and $LRMW_D$ are the muscle weights in hind leg and lumbar region, respectively, by dissection; $HLMV_{CT}$ and $LRMV_{CT}$ are the muscle volumes in hind leg and lumbar region, respectively, assessed by CT.

The measurements of $HLMV_{CT}$ and $LRMV_{CT}$ were highly repeatable between and within operators, as expected in a mainly automatic procedure. Although the operators manually defined some of the landmarks, the repeatabilities were 0.99 and 0.97 for the hind leg and the lumbar region, respectively.

3.3.1 Hind leg

Breed was the only factor that had a significant effect ($P<0.01$) on the association between

$HLMW_D$ and $HLMV_{CT}$. The estimated regression parameters, as well as the coefficients of determination (R^2) and coefficients of variation (CV) are shown in Table 3.2. The regression slopes of $HLMW_D$ on $HLMV_{CT}$ did not differ between the breeds ($P>0.05$). A common slope but different intercepts were therefore fitted. When breed was not included in the model, the intercept was 48 g ($P<0.05$) and the estimation of the slope of the regression was close to one.

Table 3.2: Estimated parameters (standard error) from the regression of muscle weight (g) on muscle volume (cm^3), coefficient of determination (R^2), residual standard deviation (r.s.d.) and coefficient of variation (CV) for the hind leg and lumbar region

Body region	Intercept (s.e.) †	Slope (s.e.) †	R^2 (%)	r.s.d.	CV (%)
HIND LEG					
Model without breed					
	48.2 (17.4) ††	0.98 (0.010)	97.4	61.5	3.7
Model including breed (common slope and different intercepts)					
TEX‡	205.9 (31.8)	0.91 (0.016)	97.7	57.7	3.5
SBF	134.4 (12.3)				
LUMBAR REGION					
Model without breed					
	88.3 (21.7)	1.05 (0.067)	83.0	32.9	7.8
Model including breed (common slope and different intercepts)					
TEX	142.4 (25.5)	0.92 (0.071)	86.0	28.9	6.9
SBF	109.5 (20.7)				
Model including breed and sex (common slope and different intercepts)					
TEX – males	112.4 (24.3)	0.95 (0.064)	88.8	26.7	6.4
TEX – females	151.3 (22.9)				
SBF – males	100.6 (19.0)				
SBF – females	106.8 (19.9)				

† All estimates were significantly different from zero ($P<0.001$), except those indicated (††significance of differences from zero ($P<0.05$).
‡TEX: Texel; SBF: Scottish Blackface

The average absolute differences between $HLMV_{CT}$ and $HLMW_D$ for the two models are shown in Table 3.3. In general, the overall average absolute differences between $HLMV_{CT}$ and $HLMW_D$, as well as the differences for each breed, were small. When different

intercepts for each breed were fitted, the average absolute difference was smaller than when a common intercept and slope were fitted in the model. Nevertheless, the maximum difference of goodness of fit between the two models was 0.4 percentage points, which was observed in the SBF lambs.

3.3.2 Lumbar region

The results for the simple regression model showed that $LRMV_{CT}$ explained 83% of the variation of $LRMW_D$ (Table 3.2). When breed, which had a significant effect ($P<0.05$), was fitted the R^2 increased to 86%. The intercepts were 142.4 and 109.5 for TEX and SBF respectively and a common slope was fitted for both breeds.

The effect of the sex of the lamb was also significant but only in TEX. The estimates of the intercepts in Table 3.2 are presented for both sexes in both breeds.

The average differences between predicted and actual $LRMW_D$ were, in general, greater than those estimated for the hind leg (Table 3.3). The improvement in the goodness of fit due to fitting breed and sex was more important in TEX, in which the average difference decreased from 7.2 to 4.8%. The average differences were smaller for SBF in the three models (5.1 to 4.3%).

Table 3.3: Average absolute differences between the predicted and actual muscle weight by dissection in the hind leg and lumbar region, as percentage of dissected muscle weight

Region	Model	Texel	Scottish Blackface	Overall
HIND LEG	Without breed	2.8%	3.4%	3.1%
	Including breed	2.6%	3.0%	2.8%
LUMBAR REGION	Without breed	7.2%	5.1%	6.2%
	Including breed	5.8%	4.3%	5.1%
	Including breed and sex	4.8%	4.3%	4.6%

3.4 Discussion

There is good evidence that CT images taken at specific anatomical positions (reference scans) provide accurate estimations of body composition in sheep (Young *et al.*, 2001a,b; Kvame and Vangen, 2006). The amount of lean, fat and bone in the carcass were predicted from three references scans by Young *et al.* (2001b) with accuracy levels of 73% to 99%

depending on breed and tissue. For total muscle weight in the carcass, the R^2 values ranged from 86% to 97% for hill sheep breeds and various terminal sire breeds, respectively (Young *et al.*, 2001b).

Tissue composition of the hind leg and the loin in lambs was investigated by Young *et al.* (2001a) using the information provided by reference scans. In the most accurate prediction equations, which always included live weight as a co-variate for both joints, the average accuracies were 95% for the prediction of the muscle weight in the hind leg and 90% in the loin. Kvame *et al.* (2004), using a similar approach reported R^2 values of 80% and 97% for the prediction of lean in the hind leg and loin, respectively.

The genetic improvement of carcass composition may require modification of the prediction equations because of changes of quantity, and possibly distribution, of the different tissues in the body. Consequently, the accuracy of the estimation of body composition and the composition of the different regions based on prediction equations derived earlier may decrease over time. The utilisation of more comprehensive approaches such as the Cavalieri scan or SCTS may provide a better alternative in the long term.

Although published reports on the utilisation of the Cavalieri approach in sheep are limited, the information available shows that this method has high accuracy. The R^2 values for the prediction of total carcass muscle from dissection based on the Cavalieri scans ranged from 95% to 97% for terminal sire breeds in the UK (Young *et al.*, 2001a). In this study, references scans and Cavalieri scans had a similar level of accuracy. Because the manual segmentation of the multiple cross-sectional images used in the Cavalieri approach is more time consuming and costly, Young *et al.* (2001a) concluded that it was not cost effective to use this approach. The reference scan approach is the main method used to provide *in vivo* predictions of carcass composition in sheep breeding programs using CT in the UK and New Zealand (Simm *et al.*, 2001; Nicoll *et al.*, 2002).

The development of automatic procedures to segment the larger number of CT images per animal used in the Cavalieri approach may make it possible to reduce costs and labour and improve cost-effectiveness. However, the random selection of the images along the body in each animal, which is inherent to this approach, makes this task much more difficult. Although the SCTS comprises a much larger number of cross-sectional images, it was possible to standardise segmentation paths for the different regions of the body (Figures 3.3

and 3.4).

We know of no previous studies on the utilisation of SCTS to calculate tissue volume in farm animals. The accuracy of the estimations of muscle volume in the hind leg and lumbar regions were determined by comparing the results obtained in lambs that were CT scanned immediately before slaughter with dissection data. Taking into account that weight is equal to the product of volume and density, and that the density of muscle is close to 1 g/cm^3 (i.e. 1.04 g/cm^3, Nord and Payne, 1995; 1.06 g/cm^3, Payne *et al.*, 2005;), the magnitude of volume and weight are expected to be very similar. The comparison of the averages of $HLMW_D$ and $HLMV_{CT}$ shows that the differences were 1% of the $HLMW_D$ in both breeds. On the other hand, $LRMV_{CT}$ was 75% of the $LRMW_D$ on average for TEX and SBF. This is explained by the fact that the segmentation in the lumbar region isolates the muscle mass that corresponds to the *m. longissimus lumborum* and *m. multifidus* (Figure 3.4d) and not all of the muscles in the joint. Results from other trials at the University of Bristol have indicated that the weights of these two muscles represent between 65 and 72% of the total lumbar muscle (A.V. Fisher, personal communication). In addition to comprising a very important proportion of the muscle mass in the lumbar region, these two muscles seem to play an important role in the definition of carcass quality traits, such as conformation and muscularity. The assessments of conformation in both live animals and carcasses (Kempster *et al.*, 1982) take into account the shape of these muscles. The segmentation of all muscles in this region, although desirable in order to obtain the meat yield for this region, also imposes difficulties for the automation of image analysis. Therefore, the isolation in the SCTS of the areas corresponding to the *m. longissimus lumborum* and *m. multifidus* was prioritised.

The results of this study show that the accuracy of estimation of muscle weight in the hind leg from SCTS (R^2=97.7%; Table 3.2) is similar to the values found by Young *et al.* (2001a) and higher than those reported by Kvame *et al.* (2004) using prediction equations based on reference scans and live weight. The intercepts of the regression of $HLMW_D$ on $HLMV_{CT}$ were significantly different for the two breeds, when breed was fitted. If $HLMV_{CT}$ is used as a direct estimator of muscle weight (in the model without breed), the predicted $HLMW_D$ will be affected by a constant value equivalent to the estimated intercept. Although the estimation will be biased, the magnitude of the bias is constant, which does not affect the prediction accuracy within a breed. If the general equation with the slope of 1 is used for both breeds, the prediction will tend to underestimate $HLMW_D$ in TEX and overestimate it in SBF, but the bias is small. Although the R^2 value decreased and the difference between predicted and

dissected muscle weight increased slightly when breed was removed from the regression model (Table 3.3), the effects on both parameters were smaller than 1%. In summary, these results indicate that $HLMW_D$ can be estimated directly from $HLMV_{CT}$ with a high degree of accuracy.

In the case of the lumbar region, the new algorithm segments the area of the *m. longissimus lumborum* (the main muscle in this region) and the *m. multifidus*, allowing the direct measurement of their volume. Although the dissected weights of these particular muscles were not available, the relationship with $LRMW_D$ indicated that there was a strong association between both measurements (R^2=83%). The comparison of different groups of muscles and the lower number of dissected weights available for the evaluation of the accuracy in the lumbar region (Table 3.1) may have contributed to the lower R^2 values for $LRMV_{CT}$ compared to $HLMV_{CT}$. The procedure used for muscle segmentation was the same in both regions, except for the number of landmarks used. The number of landmarks was related to the number of sub-regions with different shapes that required different segmentation paths. In the hind leg, four sub-regions were identified in which the segmentation paths needed to be different in order to correctly segment the areas of interest. No sub-regions were defined and one segmentation path was used within the lumbar region, as the shapes of the areas to be segmented were very similar. Consequently, an increase of the accuracy of the muscle calculation was not expected by sub-dividing the lumbar region.

Breed and sex had a more important effect on the goodness of fit for the lumbar region than for the hind leg. The data recorded in this sample suggest differences in muscle distribution within the lumbar region between TEX females and males. Although $LRMV_{CT}$ was 75% of the $LRMW_D$ on average for both breeds (Table 3.1), the results suggest that a greater proportion of the lumbar region muscle was explained by the *m. longissimus lumborum* and *m. multifidus* in the TEX males than in the females of the same breed. The difference between sexes in TEX implies that specific equations should be used for the two sexes in this breed to predict $LRMW_D$. However, this difference between sexes may be due to the sampling and requires further investigation.

The assessments in the live animal of characteristics related to carcass composition and carcass quality are particularly relevant for the genetic improvement of these traits. The possibility of measuring them *in vivo* implies that selection candidates can be evaluated directly, without the need for implementing progeny tests to estimate their merit. The

benefits of using CT in selection for improvement of carcass composition have been reported in several studies (Simm *et al.*, 2001; Nicoll *et al.*, 2002; Kvame and Vangen, 2006). Although the effect of breed was significant in the evaluation of the accuracy of the muscle mass using SCTS, the improvement was relatively small. Furthermore, this effect is less relevant in the possible utilisation for selecting within breed. The measurement of muscle volume using SCTS gives the opportunity of exploring new measurements of other relevant carcass quality traits, such as muscularity and lean yield for the different regions of the carcass that may have different economic value (Kvame *et al.*, 2004).

3.5 Conclusions

In summary, the image analysis procedure that was developed for SCTS is an accurate tool to measure *in vivo* the muscle volume in the hind leg and lumbar region of lambs. Results of this study indicate that in the case of the hind leg the CT results can be used as a direct estimator of the muscle weight in this joint. For the lumbar region, the current software measures the volume of the *m. longissimus lumborum* and *m. multifidus*, which can be used as an indicator of the total muscle weight in this region.

The automatic procedure described here allows a rapid analysis of the large number of cross-sectional images contained in SCTS with high repeatability. It provides accurate information on the muscle mass in different regions, which is needed for exploring new *in vivo* assessments of muscularity and carcass value based on meat yield. Both traits could be incorporated into sheep selection programmes based on direct measurements of the selection candidates.

Chapter 4: Accuracy of *in vivo* muscularity indices measured by computed tomography and their association with carcass quality in lambs

4.1 Introduction

In Europe, the carcass classification system for lamb carcasses, which is known as the 'SEUROP grid', is based on fat cover and conformation (CEC, 2002). From a scientific perspective one of the shortcomings of the existing system is that desirable conformation tends to be confounded with fatness (Kempster *et al.*, 1982; Simm and Murphy, 1996; Jones *et al.*, 1999). Because any improvement of carcass shape based on conformation scores may lead to an undesirable increase in fatness, muscularity, which is independent of fatness, has recently received more attention as a preferred measure of carcass shape.

Muscularity was defined by De Boer *et al.* (1974) as the thickness of muscle relative to a skeletal dimension. Although this definition may provide a way to objectively quantify muscularity, a comprehensive measurement of muscle depth is difficult to achieve and is subject to error. This limitation was later overcome in the approach of Purchas *et al.* (1991) in which the weight of the muscle and the length of the bone are combined in a simple index that is practicable and consistent with the definition of muscularity. Although it had the advantage of providing a more robust muscularity index, the approach proposed by Purchas *et al.* (1991) could not be used in live animals, which restricted its utility in genetically improving muscularity. However, computed tomography (CT) is a technique that can be used to assess body composition. Jones *et al.* (2002) and Jones *et al.* (2004) showed that it also provides good *in vivo* measures of muscularity for the whole carcass, hind leg and loin in Suffolk, Charollais and Texel (TEX) lambs. Muscularity indices were derived from CT measurements for the carcass and loin using a prediction of total muscle weight, the depth of the *m. longissimus thoracic et lumborum* (*LTL*) at the 5th lumbar vertebra and the length of the spine. Indirect measurements of hind leg muscularity, based on the ratio of length and width of the thigh muscles, were also evaluated. The very low accuracy of an indirect measurement of the femur length did not enable the development of a muscularity index for this region. Moderate phenotypic correlations between *in vivo* and dissection measurements of muscularity were reported in these studies.

Prediction of sheep carcass composition using CT scanning has been investigated in a few countries during the last ten years and this information has been included in breeding programs in the UK, Norway and New Zealand (Nicoll *et al.*, 2002; Kvame and Vangen, 2006; Macfarlane *et al.*, 2006). However, there have been advances in CT technology. Spiral CT scanning is a development that allows comprehensive assessment of specific anatomical regions or of the whole body (Imaginis, 2005). Detailed information contained in the spiral

CT scan (SCTS) has enabled new assessments of relevant compositional traits to be explored. Navajas *et al.* (2006a) (Chapter 3) reported very accurate measurements of muscle mass in the hind leg and lumbar region using automatic procedures for rapid and repeatable image analysis of SCTS. In addition, three-dimensional reconstruction of the SCTS of the body regions allows measurement of the real dimension of skeletal structures such as the femur bone to be made.

In vivo muscularity measurement by CT has only been investigated in terminal sire breeds, although relevant research using CT scanning was also carried out in the Scottish Blackface (SBF) sheep, which is the most numerous UK hill breed, with a predominantly maternal role (Young *et al.*, 2001; Lambe *et al.*, 2006). Hill breeds play an important role in lamb production in the UK, due to the stratified nature of the UK sheep industry (Dewar-Durie, 2000). As the value of lambs from these systems also depends on the current carcass payment system in the UK, conformation and fatness were included in the breeding objectives for this breed (Conington *et al.*, 2001). The contribution of *in vivo* muscularity indices in SBF needs to be investigated, due to its association with conformation.

The aims of the present study were to:
(i) assess the accuracy of the new measurement of femur length using SCTS;
(ii) investigate the accuracy of new muscularity indices for the hind leg and lumbar region developed using the muscle volumes from the SCTS in these regions (Navajas *et al.*, 2006a) (Chapter 3) and the length of the relevant skeletal structures, with reference to CT indices and indirect measurements of muscularity previously proposed by Jones *et al.* (2002);
(iii) study the association of muscularity assessed in the live animal with carcass quality in lambs from two divergent breeds that are of economic importance in the UK (TEX and SBF).

4.2 Materials and methods

Data were recorded on 120 TEX and 120 SBF lambs that were born in 2003 and 2004 and grazed in mixed-breed groups from birth to finishing (defined as commercial slaughter point, based on condition score and live weight, detailed by Navajas *et al.*, 2006a, Chapter 3). Both entire males and females were recorded. Lambs were finished in five batches in 2003 and six batches in 2004, with each batch representing lambs from each breed and sex. Lambs were CT scanned at finishing and slaughtered 4 days later. The procedures of stunning and slaughter of animals reported in this work were performed under the supervision of an

Official Veterinary Surgeon representing the UK Meat Hygiene Service and were in compliance with the legislated requirements.

4.2.1 CT scanning and measurements

Lambs were CT scanned lying on their backs in a cradle, with hind legs extended and restrained after receiving a low dose of the sedative (Rompun™; 0.1-0.2 mg xylazine hydrochloride / kg body weight). The following CT scans were taken on each lamb:

1. Topogram: ventro-dorsal view of the body of the animal;
2. Cross-sectional CT scans: images positioned at the end of the ischium bone (ISC), the 5th lumbar vertebra (LV5) and the 8th thoracic vertebra (TV8);
3. SCTS: in which multiple contiguous cross-sectional scans of a known thickness (8 mm) were collected from the proximal third of the tibia to the 4th-5th cervical vertebra (Navajas *et al.*, 2006a, in Chapter 3). The SCTS of an average lamb of 35 kg weight included approximately 110 cross-sectional images and required 3 minutes to be collected.

4.2.1.1 Spine length

The procedure used to measure the spine length from the topogram was previously described by Jones *et al.* (2002). Separate measurements of the lengths of thoracic and lumbar sections were taken. The length of the thoracic section of the spine (SPL_{Thor}) was measured as the distance from the first intervertebral disc caudal to the last rib to the first intervertebral disc cranial to the first rib. For the lumbar region, the length (SPL_{Lum}) was measured from the intervertebral disc on the cranial side of the pelvis to the first intervertebral disc caudal to the last rib. Overall spine length (SPL_{CT}) was calculated as the sum of the lengths of the two sections.

4.2.1.2 Femur length

A new method to assess the length of this bone was developed. The SCTS allows the simultaneous visualisation of the animal body in three planes (Figure 4.1). Initially the x, y and z planes are shown, enabling the scanned area to be seen longitudinally, laterally and cross-sectionally. These planes can be moved and rotated, allowing parts of the body that are in a different plane (i.e. at an angle) to be visualised. In the SCTS, the femur does not appear completely in any one of the x, y or z planes (Figure 4.1a). However, a complete view of the femur bone in the cross-sectional image is obtained by moving the y-plane from the centre of the body to where the femur can be seen and rotating the z-plane to line up with the femur as seen in the lateral view (Figure 4.1b). Consequently, the cross-sectional view shows the

whole bone. The length of the femur was measured from the intercondyle to the trochanteric fossa (Figure 4.1b) on both right and left sides and averaged (FL_{CT}).

4.2.1.3 Muscle volume in hind leg and lumbar region

The muscle volumes of the hind leg (HL) and lumbar region (LR) were obtained from the automatic image analysis of the SCTS as explained by Navajas *et al.* (2006a) (Chapter 3). These comprise all muscles in the case of the HL but only *LTL* and *m. multifidus* in the LR. Because muscle density is close to $1 g/cm^3$, the volume of these muscles was considered equivalent to their weights (for LR, $LRMW_{CT}$; for HL, $HLMW_{CT}$).

4.2.1.4 Carcass muscle weight (CMW_{CT})

CMW_{CT} was predicted from the muscle areas in the reference scans at ISC, LV5 and TV8, and live weight at CT scanning. Young *et al.* (2001) reported values for the coefficient of determination (R^2) of around 97% (residual standard deviation (r.s.d.) = 0.61 kg) between the prediction of lean weight and dissected lean weight in terminal sire breeds, whilst the accuracy for hill breed lambs was 86% (r.s.d. = 0.39 kg).

4.2.2 Dissection measurements

Carcass conformation (CONF) and fatness (FATS) were visually assessed using 15-point scales (De Boer *et al.*, 1974) and the cold carcass was weighed (CCW). The lengths of the carcass side and spine lengths were measured in both sides and averaged. The side length (SL_D) was defined as the distance from the cranial end of the symphysis pubis to the cranial dorsal edge of the first thoracic vertebra. The spine length (SPL_D) was the distance between the caudal edge of the last lumbar vertebra to the cranial edge of the first thoracic vertebra.

Each carcass side was split into five joints: fore leg (FL), neck and thorax (NT), abdomen (AB), lumbar region (LR) and hind leg (HL). With the exception of the HL, joints were dissected in 20% of the lambs, which were sampled randomly within batch/breed/sex groups. The joints were separated into muscle, subcutaneous fat, intermuscular fat and bone (Fisher and De Boer, 1994).

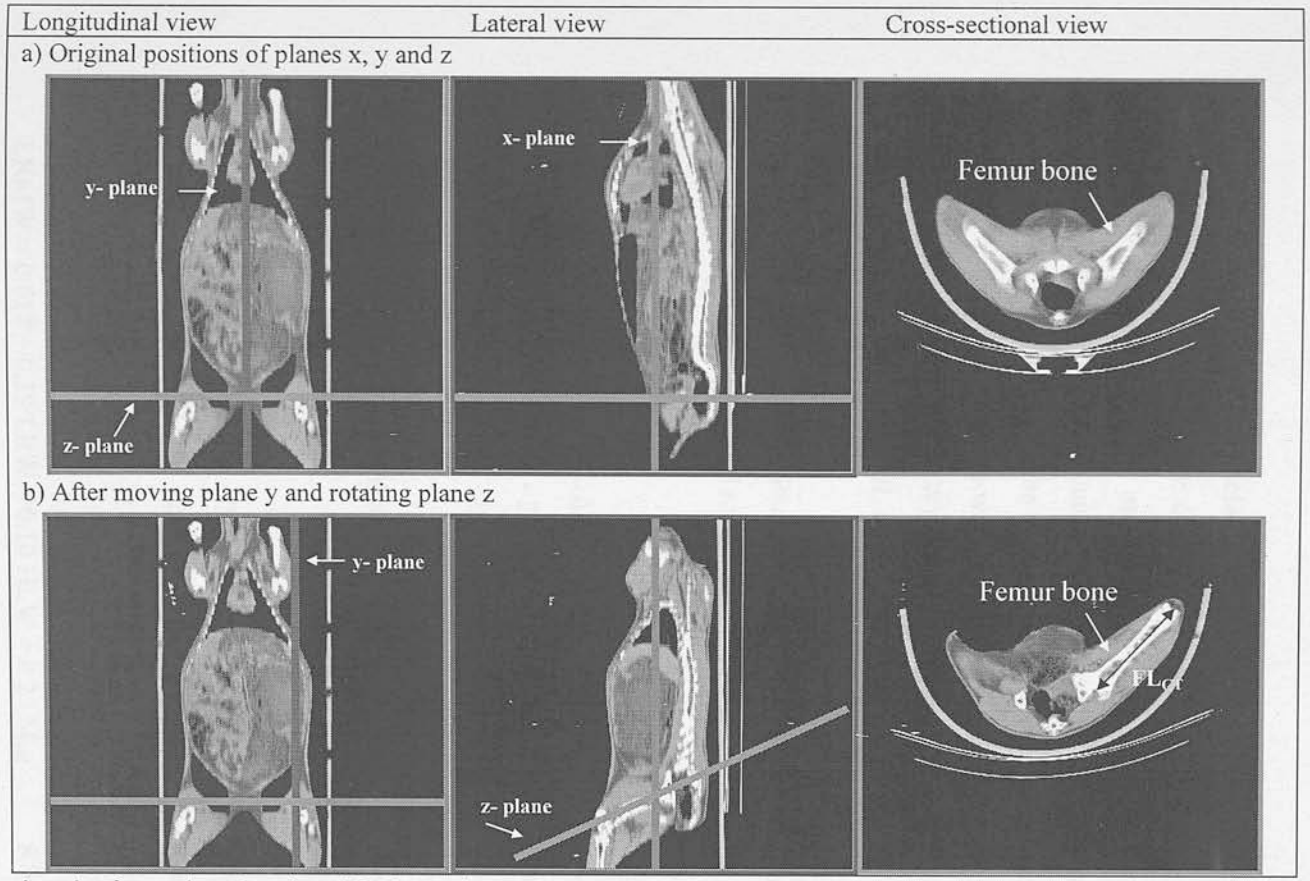


Figure 4.1: Measuring the femur length using SCTS: a) shows the longitudinal, lateral and cross-sectional views of the body of a lamb, which result from the planes x, y and z, respectively; b) the same views after moving plane y and rotating plane z to line up with the femur, as seen in the lateral view. The longitudinal view does not change because plane x remains in the same position as in a). The cross-sectional image shows the whole length of the femur bone, enabling the measurement of the actual length of the bone (FL_{CT})

The HL joints of all animals were dissected into the same tissue/depots as the other joints. For HL, the individual weights of the main muscles were also recorded (*gracilis, gluteobiceps, semitendinosus, semimembranosus, vastus lateralis* and *rectus femoris*). The length of the femur (FL_D) was measured from the head of the femur to the medial condyle at dissection, using calipers, in the animals slaughtered in 2004.

The absolute weights of muscle, fat and bone were calculated for each joint and for the side by adding the absolute values obtained from all the joints. The information available on the fully dissected carcasses was used to derive prediction equations for the carcass composition (carcass fat weight (CFW), muscle weight (CMW) and bone weight (CBW)) and weight of muscle in LR (LRMW) for the remaining animals from the composition of the HL. Multiple regression prediction models were evaluated (Genstat, 2004), including carcass side weight (CSW) or weight of LR (LRW), weight of the HL (HLW) and weights of muscle (M_{HL}), fat (F_{HL}) and bone (B_{HL}) in the HL as predictors (see Appendix 2).

The resulting equations for tissue weights in the carcass and muscle weight in LR are given below, as well as their R^2 and r.s.d. value in kg, for both breeds:

TEX carcasses:

$CFW = 0.005 + 0.199\ CSW - 0.622\ HLW + 4.309\ F_{HL}$	R^2=89.1%	r.s.d=0.15
$CMW = 0.008 + 0.578\ CSW - 2.879\ F_{HL} + 0.813\ M_{HL}$	R^2=96.8%	r.s.d.=0.13
$CBW = -0.034 + 0.101\ CSW - 0.141\ HLW + 2.146\ B_{HL}$	R^2=81.2%	r.s.d.=0.09
$LRMW = -0.008 + 0.57\ LRW - 0.12\ HLW + 0.21\ M_{HL}$	R^2=88.9%	r.s.d.=0.04

SBF carcasses:

$CFW = -0.032 + 0.288\ CSW - 0.703\ HLW + 3.906\ F_{HL}$	R^2=95.6%	r.s.d.=0.13
$CMW = 0.043 + 0.522\ CSW - 2.291\ HLW + 3.531\ M_{HL}$	R^2=94.2%	r.s.d.=0.13
$CBW = 0.015 + 0.120\ CSW - 0.356\ HLW + 2.735\ B_{HL}$	R^2=83.0%	r.s.d.=0.07
$LRMW = 0.012 + 0.39\ LRW - 0.10\ HLW + 0.25\ M_{HL}$	R^2=86.6%	r.s.d.=0.03

Total weights (dissected and predicted from HL dissection) of fat (CFW_D), muscle (CMW_D) and bone (CBW_D) in the carcass were included as carcass quality traits, as well as the muscle to bone ratio (CMW_D/CBW_D; M:B).

4.2.3 Muscle shape and muscularity indices

4.2.3.1 Muscle shape in the hind leg and lumbar region

The following linear measurements were taken in the ISC scans to calculate the hind leg muscle area shape (HLS_{CT}) as proposed by Jones *et al.* (2002): the width of the hind leg muscle was measured from the centre of the ischium bone to the farthest point of the leg shown in this scan, passing through the femur (W_{ISC}); the depth was defined as the length of a straight line from the medial to the lateral muscle boundary, passing through the popliteal fat depot and crossing the W_{ISC} line at 90° (D_{ISC}); and the thickness of the popliteal fat depot (P_{ISC}). The HLS_{CT} was computed as the ratio of (D_{ISC} minus P_{ISC}) to W_{ISC}, multiplied by 10 and averaged over both legs.

The ratio between the depth of the *LTL* (B_{CT}) in the LV5 scan (average over both sides of the animal) and the spine length was also computed as a measure of muscularity of the LR. The results reported by Jones *et al.* (2004) identified this index as the best indicator of muscle shape in the lumbar-thorax region.

4.2.3.2 Muscularity indices

Muscularity indices were calculated using *in vivo* and dissection information for the HL, LR and carcass based on the approach proposed by Purchas *et al.* (1991). Table 4.1 summarises the calculation and abbreviations of indices and indirect measurements of muscularity included in this study.

4.2.3.2.1 Hind leg muscularity

The *in vivo* muscularity index for the HL ($HLMI_{CT}$) was calculated with $HLMW_{CT}$ obtained from the SCTS (divided by 2 as SCTS measured the total for both legs), relative to the femur length (FL_{CT}).

Two dissection muscularity indices for the HL were computed. In the first index ($3MI_D$), the weights of the three main muscles around the femur (*semimembranosus*, *semitendinosus* and *gluteus biceps*; $3MW_D$) were added and the sum expressed relative to FL_D. The second post-mortem muscularity index ($HLMI_D$) included the total weight of all the HL muscles in one carcass side, which was also expressed relative to FL_D.

4.2.3.2.2 *Lumbar region muscularity indices*

Two *in vivo* muscularity indices for the LR were obtained by expressing $LRMW_{CT}$ relative to SPL_{Lum} and to SPL_{CT}. For the calculation of the LR dissection muscularity index, $LRMW_D$ (multiplied by 2 as measured via dissection only on a half carcass) was expressed relative to SPL_D.

4.2.3.2.3 *Carcass muscularity indices*

The *in vivo* (CMI_{CT}) and dissection (CMI_D) muscularity indices for the carcass were derived from the CMW_{CT} and CMW_D, which were expressed relative to SPL_{CT} and SL_D, respectively.

4.2.4 *Statistical analysis*

The distributions of the muscularity indices and M:B were checked, because traits derived from the ratio of two variables may not be normally distributed (e.g. Koerhuis and Hill, 1996). Means and medians of raw data were compared, coefficients of skewness and kurtosis calculated and the distributions checked using an empirical test (Genstat, 2004). No parameters showed substantial deviations from normality.

Simple correlations were calculated among the *in vivo* and dissection measurements of the muscle mass and skeletal dimension used to compute the muscularity indices, and between these indices and indirect measurements of muscularity. The correlations between the residuals of these traits after adjusting for CCW were also calculated. Both simple and residual correlations were calculated for each breed and these were then pooled across breeds (r-values were weighted by their corresponding degrees of freedom) for the comparison between dissection and *in vivo* assessments of muscularity.

Correlations were also calculated between muscularity and carcass quality traits. Due to significant differences in these traits between the breeds, the correlations among these variables and with muscularity indices are shown as breed-specific values. Comparisons between correlation coefficients were performed using procedure 3/61/1321 (Rasch *et al.*, 1978), which is based on the z-transformation and the u-test (for further details see Cohen 1969).

Table 4.1: Abbreviations and equations to calculate CT and dissection muscularity indices, and indirect measurements of muscularity

Abbreviations	Calculations	Description of components
HIND LEG		
$3MI_D$	$\left(\sqrt{3MW_D/FL_D^3}\right)\times 10$	$3MW_D$: weight of *semitendinosus*, *semimembranosus* and *gluteus biceps* (g); FL_D: dissection femur length (cm)
$HLMI_D$	$\left(\sqrt{HLMW_D/FL_D^3}\right)\times 10$	$HLMW_D$: muscle weight of the HL (g)
HLS_{CT}	$((D_{ISC}-P_{ISC})/W_{ISC})\times 10$	D_{ISC}: muscle depth (mm); P_{ISC}: thickness of the popliteal fat depot (mm); W_{ISC}: muscle width (mm), all measured in the ISC scan
$HLMI_{CT}$	$\left(\sqrt{HLMW_{CT}/FL_{CT}^3}\right)\times 10$	$HLMW_{CT}$: HL muscle weight by CT (g) FL_{CT} femur length by CT (cm)
LUMBAR REGION		
$LRMI_D$	$\left(\sqrt{LRMW_D/SPL_D^3}\right)\times 10$	$LRMW_D$: LR muscle weight by dissection (g) SPL_D: spine length of carcass (cm)
$LTLMI_{CT}$	$(B_{CT}/SPL_{CT})\times 10$	B_{CT} depth of the *LTL* muscle by CT (cm) SPL_{CT}: spine length by CT (cm)
$LRMI_{CT}$	$\left(\sqrt{LRMW_{CT}/SPL_{CT}^3}\right)\times 10$	$LRMW_{CT}$; LR muscle weight by CT (g) SPL_{CT} total spine length by CT (cm)
$LRMI_{CTL}$	$\left(\sqrt{LRMW_{CT}/SPL_{Lum}^3}\right)\times 10$	$LRMW_{CT}$; LR muscle weight by CT (g) SPL_{Lum}: length of lumbar spine by CT (cm)
CARCASS		
CMI_D	$\left(\sqrt{CMW_D/SL_D^3}\right)\times 10$	CMW_D: carcass muscle weight by dissection (g) SL_D; carcass side length (cm)
CMI_{CT}	$\left(\sqrt{CMW_{CT}/SPL_{CT}^3}\right)\times 10$	CMW_{CT}: carcass muscle weight predicted from CT (g) SPL_{CT} total spine length by CT (cm)

Because FL_D was only measured in the lambs slaughtered in 2004, the relationship of the *in vivo* and dissection measurement for the HL was investigated only in this data set (data set

1). Data recorded in 2003 and 2004 were considered (data set 2) for the investigation of the associations among all *in vivo* muscularity indices and with carcass quality traits.

4.3 Results and discussion

4.3.1 Summary statistics

Table 4.2 shows the means and standard deviations (s.d.) of *in vivo* and dissection muscularity indices and carcass quality traits. The differences between the breed means were significant ($P \leq 0.05$) for all traits in this table. SBF were on average 2 kg lighter at slaughter than TEX lambs and CCW reflected this difference. TEX carcasses were leaner than SBF, as indicated by the average FATS and CFW_D. A higher average CONF was allocated to the TEX carcasses, which also had higher average M:B. TEX had higher average values for the muscularity indices of the HL, LR and carcass. The differences between the breeds were quite similar in the different regions. The range of these differences, expressed as percentage of the value in TEX, was between 15% (LR and HL) and 19% for the whole carcass.

However, the difference between breeds was not significant for FL_{CT} and FL_D ($P>0.05$). The means (s.d.) were 15.5 cm (0.48), and 14.2 cm (0.45) for FL_D and FL_{CT}, respectively, with the difference between these means due to the different landmarks used in the measurement by CT and after dissection.

4.3.2 Accuracy of CT muscularity indices

Considerable information is now available on the utilisation of CT in sheep to predict total carcass composition (Nicoll *et al.*, 2002; Macfarlane *et al.*, 2006) and the distribution of muscle and fat (Kvame and Vagen, 2006) in the main joints, and internal (non-carcass) fat depots (Lambe *et al.*, 2006). Nevertheless, the study by Jones *et al.* (2002) is the only one to date on the development of muscularity measurements using this technology. Based on the accuracy and the parameters estimated by Jones *et al.* (2004), muscularity indices for the LR ($LTLMI_{CT}$) and carcass (CMI_{CT}) were proposed. These indices were computed according to the approach proposed by Purchas *et al.* (1991). An indirect measure of the muscularity in the HL (HLS_{CT}) was also suggested (Jones *et al.*, 2002) as an alternative method to quantify the shape of the muscle in this region, because of the low accuracy ($R^2<5\%$) obtained for the measurement of the femur length from CT in their study. The very low association arose because it was impossible to account for the variation of leg positioning in the vertical plane (Jones *et al.*, 2002).

Table 4.2: Means and standard deviations (s.d.) for weight, *in vivo* and dissection muscularity indices and carcass traits

	TEX[††]		SBF	
	Mean[§]	s.d.	Mean[§]	s.d.
Live weight (kg)[†]	37.4	3.8	34.8	3.5
CCW (kg)	17.1	2.5	14.4	2.1
MUSCULARITY				
Lumbar region				
$LRMI_D$	0.95	0.07	0.80	0.05
$LTLMI_{CT}$	0.64	0.07	0.53	0.07
$LRMI_{CTL}$	3.16	0.38	2.66	0.35
$LRMI_{CT}$	0.86	0.07	0.73	0.07
Hind leg				
$3MI_D$[‡]	4.18	0.26	3.41	0.21
$HLMI_D$[‡]	7.09	0.37	5.84	0.30
HLS_{CT}	5.79	0.64	4.23	0.66
$HLMI_{CT}$	8.06	0.45	6.79	0.47
Carcass				
CMI_D	2.47	0.11	2.00	0.09
CMI_{CT}	3.46	0.24	2.79	0.16
CARCASS CLASSIFICATION				
CONF	9.38	1.49	5.60	1.61
FATS	3.09	1.93	5.97	2.22
CARCASS COMPOSITION				
CMW_D (kg)	10.90	1.25	7.78	1.01
CFW_D (kg)	2.44	0.95	3.10	1.00
M:B	3.94	0.36	3.05	0.30

[†] All traits, expect those indicated, were recorded in 120 TEX and 120 SBF lambs slaughtered in 2003 and 2004 (data set 2).
[‡] Traits calculated for 58 TEX and 74 SBF lambs slaughtered in 2004 because the dissection femur length was only available for these lambs (data set 1).
[§] The differences between the breed means are significant ($P \leq 0.05$) for all traits.
[††] TEX: Texel; SBF: Scottish Blackface

The accuracy of *in vivo* measures of muscularity was evaluated based on their relationship with equivalent measurements derived from post-slaughter data, which are presented in

Table 4.3. Results are compared with associations reported in other studies, although these should be interpreted with caution due to possible differences in the variation of the traits of interest among studies.

4.3.2.1 Carcass muscularity

The approach used to assess *in vivo* carcass muscularity (CMI_{CT}) in the current study was the same as used by Jones *et al.* (2002). However, in the case of the CMI_D, carcass muscle weights were obtained by the full dissection of all carcasses by Jones *et al.* (2002), whilst in this study CMW_D was predicted for 80% of the carcasses from the composition of the HL. Nevertheless, the correlation between CMI_D and CMI_{CT} was 0.55 in both cases. This may be explained by the similar accuracies of the *in vivo* measurement of the spine and prediction of the muscle weight by CT (Macfarlane *et al.*, 2006), in addition to the accurate prediction of carcass composition using the tissue composition of the hind leg described above in the Material and Methods section (TEX, R^2=96.8%; SBF, R^2=94.2%).

Table 4.3: Simple (and residual[†]) correlation coefficients between *in vivo* and dissection muscularity indices (pooled over breeds)

HIND LEG[‡]	$3MI_D$	HLS_{CT}	$HLMI_{CT}$
HLS_{CT}	0.53 (0.45)	--	
$HLMI_{CT}$	0.85 (0.81)	0.54 (0.48)	--
$HLMI_D$	0.97 (0.96)	0.51 (0.45)	0.89 (0.86)
LUMBAR REGION[§]	$LTLMI_{CT}$	$LRMI_{CTL}$	$LRMI_{CT}$
$LRMI_{CTL}$	0.62 (0.50)	--	
$LRMI_{CT}$	0.76 (0.67)	0.72 (0.62)	--
$LRMI_D$	0.44 (0.33)	0.46 (0.37)	0.55 (0.47)
CARCASS[§]	CMI_D		
CMI_{CT}	0.55 (0.50)		

[†] after adjusting for carcass weight; [‡] Data set 1, n=132; [§] Data set 2, n=240

4.3.2.2 Hind leg muscularity

The correlation coefficient between $HLMI_D$ and $3MI_D$ was close to 1 in TEX and SBF (Table 4.3). Although $HLMI_D$ includes all muscles in the HL, unlike $3MI_D$ which comprises only those that surround the femur, the very high correlation coefficient between the indices for both breeds indicates that $HLMI_D$ is as accurate as $3MI_D$ in assessing muscularity.

The relationships between $HLMI_{CT}$ and the dissection muscularity indices were very strong (Table 4.3). However, the correlations between the HLS_{CT} with $HLMI_D$ and $3MI_D$ were moderate. Although the correlation between HLS_{CT} and $3MI_D$ in this study was lower than the one reported by Jones *et al.* (2002), the difference was not significant (0.53 in this study *vs* 0.63 calculated by Jones *et al.* (2002); $P>0.05$).

The high accuracy of $HLMI_{CT}$ is explained by the strong association between *in vivo* and post-slaughter assessments of the components of the indices. The R^2 between muscle mass determined by CT and dissection was 97.7%. This value, as well as the regression coefficients for $HLMW_D$ *vs* $HLMW_{CT}$ ($HLMW_D = 48.7 + 0.99xHLMW_{CT}$) were in agreement with those reported by Navajas *et al.* (2006a) (Chapter 3).

The length of the femur determined using the SCTS (FL_{CT}) in the present study provides a very accurate measurement of the length of the bone measured at dissection (R^2=87.0%; Table 4.4). The simple and residual regression coefficients between FL_{CT} and FL_D were 0.93 and 0.91, respectively. The inclusion of the effect of breed in the regression model increased the R^2 value only slightly by 0.7 percentage points and the regression coefficients were similar ($P>0.05$). This confirms that CT can be used to directly assess the femur length without requiring adjustment for breed. The availability of accurate measures of femur length used in the calculation of $HLMI_{CT}$ had an important effect in improving the accuracy of the new index compared to HLS_{CT}.

Table 4.4: Estimated parameters from the regression of femur length from dissection (FL_D; cm) on corresponding length from CT (FL_{CT}; cm), coefficient of determination (R^2) and residual standard deviation (r.s.d.)

FL_D *vs* FL_{CT} [†]		α [‡]	β [‡]	R^2 (%)	r.s.d
Simple regression model		1.63	0.98	86.8	0.18
Regression model	TEX	1.91	0.96	87.5	0.17
including breed[††]	SBF	1.82			

[†] Data recorded in 58 TEX and 74 SBF lambs slaughtered in 2004.
[‡] Standard error of the estimates were 0.47 and 0.01 for α (intercept) and β (regression slope), respectively.
[††] TEX: Texel; SBF: Scottish Blackface

4.3.2.3 *Lumbar region muscularity*

The associations between the *in vivo* muscularity indices for the LR ($LTLMI_{CT}$, $LRMI_{CT}$ and $LRMI_{CTL}$) ranged from 0.62 to 0.76 (Table 4.3). The correlation coefficients between these indices and $LRMI_D$ were lower (0.44 to 0.55). The highest correlation was between $LRMI_D$ and $LRMI_{CT}$.

Following the same approach than for $HLMI_{CT}$, $LRMI_{CT}$ was calculated using $LRMW_{CT}$ (Navajas *et al.*, 2006a, in Chapter 3) and SPL_{CT} from the topogram (Jones *et al.*, 2002). These measurements were less accurate than the assessment of muscle mass and bone length in the HL and consequently the accuracy of $LRMW_{CT}$ was somewhat lower than the one for the HL (Table 4.3).

The correlation coefficients between SL_D and SPL_{CT}, SPL_D and SL_D, and SPL_{CT} and SPL_D were all moderate to high, with values in the range of 0.60 to 0.72 (Table 4.5). The association between SPL_{Lum} and SPL_{Thor} and SPL_D and SL_D were low to moderate. For the muscle component of the index, the correlations between $LRMW_{CT}$ and $LRMW_D$ were 0.87 and 0.90 for TEX and SBF, respectively.

Table 4.5: Simple (and residual[†]) correlation coefficients between carcass side and spine lengths assessed *in vivo* and post-slaughter (pooled over breeds)

	SL_D	SPL_D	SPL_{CT}
SPL_D	0.72 (0.53)		
SPL_{CT}	0.67 (0.55)	0.63 (0.61)	
SPL_{Lum}	0.36 (0.20)	0.39 (0.40)	0.61 (0.57)
SPL_{Thor}	0.51 (0.45)	0.38 (0.21)	0.70 (0.61)

[†] after adjusting for carcass weight

Although good *in vivo* assessments can be obtained for the LR, further improvements in measurements of both components, in particular for the spine length, may also improve the accuracy of the corresponding muscularity index.

4.3.3 Association among CT muscularity indices

The correlation between $LTLMI_{CT}$ and $LRMI_{CT}$ (0.76) was higher than between HLS_{CT} and $HLMI_{CT}$ (0.61) (Table 4.6). In general, the association among indices and indirect

measurements of muscularity for the HL and LR were moderate (range: 0.46 – 0.62). However, the association between LR muscularity and CMI_{CT} was stronger than the one between HL muscularity and CMI_{CT}. This may be caused by the fact that LR and carcass indices have the spine length as a common component. Besides this difference between body regions, the correlation of CMI_{CT} with muscularity assessed as the combination of muscle mass and bone length was higher than with $LTLMI_{CT}$ and HLS_{CT} (Table 4.6).

Table 4.6: Simple (and residual[†]) correlation coefficients between *in vivo* measures of muscularity (n=240, pooled over breeds)

	$LTLMI_{CT}$	$LRMI_{CT}$	HLS_{CT}	$HLMI_{CT}$
$LRMI_{CT}$	0.76 (0.67)	--		
HLS_{CT}	0.52 (0.39)	0.46 (0.31)	--	
$HLMI_{CT}$	0.62 (0.46)	0.57 (0.36)	0.61 (0.50)	--
CMI_{CT}	0.63 (0.57)	0.77 (0.76)	0.31 (0.20)	0.43 (0.31)

[†] after adjusting for carcass weight

Although the numerator of the muscularity index proposed by Purchas *et al.* (1991) is considered an approximation of muscle depth, the value of this approximation in the LR may be reduced because changes in muscle weight may be linked to changes in either muscle width or depth. Nevertheless, the high correlation between $LRMI_{CT}$ and $LTLMI_{CT}$ (Table 4.6) suggests that both provide a similar indication of the muscularity in this region. A relatively homogeneous shape may explain why a single measurement of the depth of the *LTL* muscle at one anatomical location (5^{th} lumbar vertebra) was as good as the volume of this muscle in the calculation of the index. A similar phenotypic correlation was reported by Waldron *et al.* (1992) between a dissection muscularity index equivalent to $LTLMI_{CT}$ and the muscularity of the *LTL*, which was computed using the muscle weight and carcass length.

The higher complexity of the HL shape may be one of the reasons explaining the lower association between the muscularity assessments in this region compared to the LR ($P<0.05$). Another reason to be considered is that HLS_{CT} is a combination of two linear measurements of the muscle shape but without reference to any skeletal dimension.

The correlations between the indices for the HL and LR ranged were low to moderate. The associations between CMI_{CT} and the LR muscularity indices were high but low with the HL. The magnitude of these correlations indicates that none of these indices is a perfect indicator

of muscularity of other regions at a phenotypic level. Jones *et al.* (2004) and Wolf *et al.* (2006) arrived at a similar conclusion regarding the association among muscularities of different regions in different terminal sire breeds, including TEX.

In summary, the results support the conclusion that one single measure of muscularity does not fully describe the muscularity of different regions and the whole carcass at a phenotypic level. This conclusion seems to be applicable to terminal sire breeds and hill breeds, as exemplified by the SBF in this study.

4.3.4 Association among muscularity assessed in vivo, carcass shape and composition

Defining carcass quality is complex due to the multiple characteristics that could be considered, differing in the interpretation of quality between countries, and among markets within the same country (Kirton, 1989). Two groups of carcass quality traits were considered here. The first group included CONF and FATS which are those included in the current grading scheme in the UK (CEC, 2002). The second group comprised the characteristics related to the objective determination of carcass composition such as M:B, CMW_D and CFW_D. In the analysis of relationships among the traits included in this study, the residual correlations after fitting CCW were calculated. In the case of CMW_D and CFW_D, these traits can be interpreted as the proportions of these tissues in the carcass and they will be referred to as such from here onwards. The relationships between some of the carcass traits differed between breeds. Consequently, the associations between the CONF and FATS, CMW_D and CFW_D and the *in vivo* measurements are presented separately for each breed in Table 4.7.

The M:B ratio is an objective measurement which has often been associated with superior muscularity although this association has not been very strong in all cases (Purchas *et al.*, 1991). The associations calculated in this study indicate a moderate positive relationship between M:B and muscularity indices for the LR and HL in TEX and SBF lambs. These results are in agreement with the relationships reported by Jones *et al.* (2004) and Waldron *et al.* (1992) for the LR. However, Jones *et al.* (2004) reported a low correlation between HLS_{CT} and M:B. The results in Table 4.7 show that the associations between CMI_{CT} and M:B were weak in SBF and not different from zero in TEX ($P>0.05$), whilst Jones *et al.* (2004) found correlations higher than 0.40. This stronger association may be because both traits were calculated from the CT tissue predictions.

Table 4.7: Simple (and residual[†]) correlation coefficients between CT measurements of muscularity and carcass quality traits

TEX[††]	$LTLMI_{CT}$		$LRMI_{CT}$		HLS_{CT}		$HLMI_{CT}$		CMI_{CT}	
FATS	0.30[‡]	(0.02)	0.26	(-0.01)	0.02	(-0.24)	0.13	(-0.22)	0.05	(-0.19)
CONF	0.30	(0.27)	0.21	(0.17)	0.29	(0.26)	0.50[a]	(0.51)	0.19	(0.15)
CMW_D	0.56	(0.27)	0.55	(0.34)	0.49	(0.39)	0.64	(0.48)	0.43	(0.29)
CFW_D	0.38	(-0.08)	0.29	(-0.21)	0.15	(-0.29)	0.32[a]	(-0.21)	0.11	(-0.33)
M:B	0.24	(0.24)	0.28	(0.28)	0.21[a]	(0.19)	0.33[a]	(0.34)	0.15	(0.13)
CCW	0.51	--	0.47	--	0.36	--	0.51	--	0.34	--
SBF	$LTLMI_{CT}$		$LRMI_{CT}$		HLS_{CT}		$HLMI_{CT}$		CMI_{CT}	
FATS	0.16	(-0.15)	0.25	(-0.09)	0.04	(-0.26)	0.35	(0.03)	0.04	(-0.14)
CONF	0.49	(0.30)	0.40	(0.14)	0.40	(0.21)	0.67[b]	(0.52)	0.29	(0.16)
CMW_D	0.63	(0.38)	0.68	(0.34)	0.59	(0.40)	0.69	(0.29)	0.38	(0.24)
CFW_D	0.42	(-0.08)	0.48	(-0.13)	0.36	(-0.09)	0.55[b]	(-0.05)	0.17	(-0.18)
M:B	0.43	(0.25)	0.42	(0.20)	0.45[b]	(0.30)	0.54[b]	(0.36)	0.20	(0.07)
CCW	0.55	--	0.63	--	0.48	--	0.67	--	0.31	--

[†] after adjusting for carcass weight
[‡] Coefficients with absolute values >0.17 are different from zero ($P \leq 0.05$)
[a,b] Superscripts indicate that correlation coefficients differ significantly between breeds ($P \leq 0.05$)
[††] TEX: Texel; SBF: Scottish Blackface

Improved muscularity in the HL, LR and whole carcass was associated with an increased CMW_D and higher proportion of muscle in the carcass, as indicated by the simple and residual correlations, respectively. Similar associations were found by Jones *et al.* (2004) and Waldron *et al.* (1992) for muscle weight and muscularity and by Wolf *et al.* (2006) who reported in TEX moderate positive correlations between muscle proportion and objective measures of carcass muscularity or subjective assessments of leg muscularity.

Although muscularity by definition does not include fat, it does not ensure independence from fatness due to possible indirect associations between compositional characteristics. The association between CFW_D and muscularity were positive in SBF for all the muscularity indices. For the TEX lambs, the correlations were also positive yet small between CFW_D and both LR muscularity indices and $HLMI_{CT}$, but not different from zero for HLS_{CT} and CMI_{CT} ($P>0.05$). There were differences between the breeds regarding the relationship with the

proportion of fat. When the traits were adjusted for CCW, muscularity was not related to the level of fatness in SBF, whilst the association tended to be negative in TEX. Similar results were reported by Wolf *et al.* (2006) in TEX lambs.

The shape of muscles might be defined as one component of CONF. As such, better muscularity is expected to link to better conformation. In agreement with this, the associations between objective muscularity indices and conformation were positive in both breeds (Table 4.7). In common with Abdullah *et al.* (1993) and Laville *et al.* (2004), who found that CONF was strongly influenced by leg muscularity, the estimated correlation with $HLMI_{CT}$ was stronger than with the other muscularity measures. This may be explained by the fact that HL development had a greater effect on the judgement of CONF.

Previous studies showed that there is an association between CONF and fatness (Kempster *et al.*, 1982; Simm and Murphy, 1996; Jones *et al.*, 1999), which restricts the utilisation of conformation scores as the tool to improve carcass shape. The association is because carcasses with thicker fat cover tend to be judged to have better conformation. In the present study, this was the case for SBF carcasses, in which CONF was positively correlated with FATS (0.44) and CFW_D (0.51). After adjusting for CCW, these associations remained positive and significant (FATS, 0.26; CFW_D, 0.19). On the other hand, FATS and CONF were independent in TEX ($P>0.05$), which is probably due to the leanness of this breed.

4.4 Conclusions

The results of this study suggest that improved muscularity is not associated with detrimental effects on carcass composition at a phenotypic level, after adjusting for CCW. This is particularly relevant for the terminal sire breeds, in which the economically important traits included in breeding programmes tend to be carcass composition traits. In the case of SBF, the CT muscularity indices provide an option to improve CONF, in addition to leanness, using measurements that at a constant weight are independent of fatness.

Compared to previous CT muscularity indices, the accuracy of the new index for the HL was much higher. The accurate measurement of femur length by CT described in this study, which was used in the calculation of the new HL index made an important contribution to the higher accuracy of the index. The improvement in accuracy was smaller for the LR.

In summary, the proposed CT muscularity indices for the HL and LR had high and moderate

accuracy, respectively, in the two contrasting breeds (TEX and SBF). These indices may be useful for the *in vivo* identification of selection candidates with superior muscularity in the regions of the carcass where high priced cuts are located.

Chapter 5: Muscularity and eating quality of Scottish Blackface and Texel lambs

5.1 Introduction

The genetic improvement of carcass quality traits has received much emphasis in sheep breeding programmes in the UK for several years, especially in terminal sire breeds (e.g. Texel (TEX), Suffolk, Charollais) where the breeding objective is to increase lean weight with little or no change in fat weight. Selection is based on an index combining information on live weight, ultrasound muscle depth and ultrasound fat depth as well as, in some schemes, lean and fat weight predicted using X-ray computed tomography (CT) scanning (Macfarlane, 2006). Because of the importance of maternal characteristics and hardiness in hill sheep there has been less selection pressure on carcass traits. However, subjective conformation and fat scores awarded at the abattoir are economically important traits that are included in the breeding objectives of some of the breeds, such as the Scottish Blackface (SBF) (Conington *et al.*, 2001).

One of the limitations of the existing carcass classification system used in Europe is that desirable carcass conformation tends to be associated with increased levels of fatness (Kempster *et al.*, 1982; Simm and Murphy, 1996; Jones *et al.*, 1999). However, muscularity itself, defined as the shape of the muscle, and by definition independent of fatness, is commercially important and has received recent attention as an alternative, and in some ways preferable, index of carcass shape (Jones *et al.*, 2002). In addition to the enhancement of conformation, the improvement of muscularity may also be important because the shape of the cut has an effect on the attractiveness of the meat to consumers, who tend to prefer plump leg joints and large round rather than thin chops (Chambers and Bowers, 1993; Laville *et al.*, 2004; Kukowski *et al.*, 2005).

Estimated breeding values of seedstock for muscularity are available for some breeds in the UK based on a measurement of this trait developed by Jones *et al.* (2002). This measurement is a two-dimensional (2D) approach and has been calculated as the ratio between the leg muscle width and its length measured on a cross-sectional CT scan image at the ischium (Jones *et al.*, 2002). A new method to assess muscularity as a three-dimensional (3D) trait in the hind leg and lumbar region of live animals was later developed using spiral CT scans (SCTS) (Navajas *et al.*, 2007, in Chapter 4). These muscularity indices, which were calculated by combining measurements of muscle volume and lengths of the relevant skeletal structures, were shown to correlate better with dissection values than the previous 2D assessments, particularly in the hind leg. The favourable associations of the muscularity indices, measured *in vivo*, with other carcass quality traits indicate that they may provide a

good opportunity to improve carcass quality, evaluated in terms of weights of lean and fat or conformation and fatness scores (Navajas *et al.*, 2007, in Chapter 4).

The associations between muscle shape and leanness on meat eating quality traits in sheep are unclear. Unfavourable associations of lean meat yield and muscle growth with meat quality, in particular with tenderness, were reported in the Poll Dorset breed due to the Callipyge gene, which causes a more rapid muscle accretion, and compact and leaner carcasses (Freking *et al.*, 1999). On the other hand, only mild effects of the Carwell gene (known to cause muscle hypertrophy in the *m. longissimus*) on tenderness were reported by Jopson *et al.* (2001), whilst Johnson *et al.* (2005a) found no effects of a quantitative trait loci (QTL) for increased muscling on meat quality traits in TEX lambs. Only Hopkins *et al.* (2005) and Johnson *et al.* (2005b) considered muscularity and/or muscle mass as polygenic traits. Hopkins *et al.* (2005) also reported an unfavourable effect of high estimated breeding values for muscling of Poll Dorset sires on the meat eating quality of their progeny. However, the phenotypic correlations between leg muscularity and meat quality traits estimated by Johnson *et al.* (2005b) were low and inconclusive.

In general terms, there is less information on how factors such as breed (Leymaster *et al.*, 2006), or sex (Arsenos *et al.*, 2002) influence meat eating quality, than on the effects on growth and carcass quality traits. SBF and TEX are breeds of contrasting muscularity and are significant contributors to lamb production in the UK. TEX is a very well-muscled breed that has been included in several breed comparison studies, and has been shown to have a lower carcass fat content, a higher lean meat yield, and an improved carcass and leg conformation than other terminal sire breeds, although no significant differences in meat quality characteristics were reported (Johnson, 2003). In the case of the UK hill sheep breeds, such as SBF, comparative information is limited. In a study comparing different breeds and crosses, carcass weight and conformation and fat scores were higher in TEX-sired lambs compared with purebred SBF lambs at a similar age (Carson *et al.*, 2001a). The effect of lamb genotype was significant for ultimate pH, with SBF lambs having the highest values, but meat cooking loss, sarcomere length or Warner-Bratzler shear force were not affected by genotype (Carson *et al.*, 2001b). Regarding the influence of lamb sex on eating quality, only a few studies have shown statistically significant differences between sexes (tenderness, Dransfield *et al.*, 1990, Johnson *et al.*, 2005b; flavour, Arsenos *et al.*, 2002), finding that quality in ram lambs was lower than in females, although the differences were small.

The objectives of our study were: (i) to investigate the differences in muscularity in the hind leg and lumbar region between two breeds of contrasting muscularity (TEX and SBF), between sexes and between the progeny of sires with high and low muscularity within breed, and (ii) to evaluate the effect of these factors on eating quality, assessed by taste panel, of muscle samples from two carcass regions (hind leg and lumbar region).

5.2 Material and Methods

5.2.1 Experimental animals and management

A flock of approximately 250 mixed-age ewes comprising approximately half SBF and half TEX was established at SAC. Ewes were artificially inseminated in 2002 and 2003 with semen from sires of their own breed. Sires were selected from among those that had previously been CT scanned, and used in both years. To produce lambs within each breed with increased variation in muscularity, rams were selected that were divergent for muscularity in the hind leg. Muscularity was measured using the 2D approach proposed by Jones *et al.* (2002), referred to hereafter as hind leg shape, which is based on the ratio between the leg muscle width and its length measured on a cross-sectional CT scan image at the ischium (Jones *et al.*, 2002). Sire rams were defined as high muscularity (HM) or low muscularity (LM) according to these measurements taken at previous scanning events. Five HM and five LM sires were used per breed, with approximately equal numbers of ewes in each sire group within year. The raw means and standard deviations of hind leg shape within each of these groups for each breed are shown in Table 5.1. Following AI, ewes were run with back-up rams from the same sources as the AI rams.

Table 5.1: Means (and standard deviations) of hind leg shape ratios of sire groups[‡]

Breed[†]	HM sires	LM sires	Ratio HM/LM
TEX	7.84 (0.36)	6.30 (0.27)	1.24
SBF	6.06 (0.26)	4.62 (0.15)	1.31

[†] TEX: Texel; SBF: Scottish Blackface
[‡] HM: high muscularity; LM: low muscularity

This study included information from a total of 471 ewe and ram lambs (SBF n=231; TEX n=240) that were recorded from birth to slaughter in the years 2003 and 2004, which also had CT information. SBF lambs were from 11 sires, with between 2 and 49 lambs per sire (average = 21) from an average of 16 dams per sire. TEX lambs were from 10 sires, with between 14 and 37 lambs per sire (average = 24) from an average of 17 dams per sire.

Lambs were grazed on lowland paddocks in mixed-breed groups from birth to slaughter. Although lambs were finished off grass, they were provided with supplements from autumn onwards because of the lower grass availability and quality. In order to minimise feed effects on meat quality, only dried grass pellets (2003) and haylage or hay (2004) were given. Lambs were CT scanned on a maximum of four occasions during the growing period and pre-slaughter. Lambs were slaughtered in five batches in 2003 and six batches in 2004. Selection for each batch depended on live weight and condition score. Lambs were finished at a target condition score of 3 (on a subjective scale of 0 to 5) and a minimum live weight of 35 kg in 2003 and 32 kg in 2004, due to slower growth rates in the second year. Each finishing batch was of mixed breed and sex. Age at finishing ranged from 91d to 202d, with an average of 139d. Half of the lambs in each finishing batch (balanced for breed and sex) were slaughtered at finishing. The other half of the lambs of each batch were slaughtered 30 days later, the withdrawal period for the sedative used as a pre-requisite for CT scanning, to allow taste panel analyses (SBF n=110; TEX n=119). Lambs in the second half of each batch continued to be managed to achieve positive growth until slaughter.

5.2.2 CT muscularity indices

From the pre-slaughter CT scans from all lambs, muscularity in the hind leg ($HLMI_{CT}$) and lumbar region ($LRMI_{CT}$) was calculated using previously derived CT muscularity indices (Navajas *et al.*, 2007, in Chapter 4; Table 5.2), which were based on the approach proposed by Purchas *et al.* (1991). The volumes of muscle in the hind leg ($HLMW_{CT}$) and lumbar region ($LRMW_{CT}$) were calculated using an automatic image analysis method for SCTS (Navajas *et al.*, 2006a, in Chapter 3). The length of the femur bone (FL_{CT}) was measured on a 3D reconstruction of the SCTS, whilst the spine length (SPL_{CT}) was measured on a 2D longitudinal CT image (topogram) (Navajas *et al.*, 2007, in Chapter 4).

5.2.3 Taste panel evaluation

Taste panel evaluations of *m. semimembranosus* (hind leg) and *m. longissimus lumborum* (lumbar region) muscle samples from 229 lambs were performed. These samples were removed from the right carcass side the day after slaughter, and were vacuum packed, aged for seven days (slaughter day = day 0) at 2-4 °C and then frozen at -20 °C. For the sensory evaluation, samples were thawed at 4 °C overnight. They were then cut into 2 cm thick steaks and cooked in a contact grill (George Forman Double Knockout grill, model 10635) until the internal temperature reached 75 °C, measured by a thermocouple inserted into the

geometric centre of the sample. Between 6 and 10 assessors rated 2 cm^3 samples of each muscle. The assessors used 8-point category scales, as in the study by Sañudo *et al.* (1998a), to evaluate the following traits: texture (1 – extremely tough, 8 – extremely tender); juiciness (1 – extremely dry, 8 – extremely juicy); lamb flavour intensity (1 – extremely weak, 8 – extremely strong), abnormal flavour intensity (1 – extremely weak, 8 – extremely strong) and overall liking (hedonic) (1 – dislike very much, 8 – like very much).

Table 5.2: *In vivo* muscularity indices using computed tomography (CT) †

Muscularity index	Calculations	Description of components
$HLMI_{CT}$	$\left(\sqrt{HLMW_{CT}/FL_{CT}^3}\right)\times 10$	$HLMW_{CT}$: HL muscle weight by CT (g) FL_{CT} femur length by CT (cm)
$LRMI_{CT}$	$\left(\sqrt{LRMW_{CT}/SPL_{CT}^3}\right)\times 10$	$LRMW_{CT}$; LR muscle weight by CT (g) SPL_{CT} total spine length by CT (cm)

HL: hind leg; LR: lumbar region
† Described by Navajas *et al.* (2007)

5.2.4 Statistical analysis

The following linear mixed model was used to analyse the muscularity indices:

$y = Xb + Zu + e$

where

y is the data vector of observed variables, $HLMI_{CT}$ or $LRMI_{CT}$;

b is the vector of fixed effects: year-batch, slaughter group (slaughter 4 or 30 days after CT scanning), rearing rank (single or multiple), age of dam (2 to 6 years of age), breed (SBF or TEX), sex (ram or ewe lamb) and sire group for muscularity (HM or LM). The following interactions were also fitted ('.' representing an interaction): breed.sire group + breed.sex + sex.sire group + year-batch.slaughter group + year-batch.breed + year-batch.sire group + year-batch.sex + rearing rank.siregroup + rearing rank.sex;

u is the vector of random sire effects;

e is the vector of random residuals, and

X and Z are the matrices of incidence associated with vectors b and u, respectively.

A mixed model was also used to analyse the eating quality traits. The fixed effects were as described above, except for slaughter group (and the interactions including this effect), which was not included because taste panel assessment was only performed on samples from

the animals slaughtered 30 days after CT scanning. The random effects were sample identification, date of assessment, assessor and sire. The direct effect of muscularity was also assessed independently for each breed, by replacing sire group for muscularity in the previous model by the muscularity index for the specific carcass region, which was fitted as a co-variate.

All models were fitted using REML in Genstat (Payne *et al.*, 2006).

5.3 Results

Table 5.3 shows the adjusted means of $HLMI_{CT}$ and $LRMI_{CT}$ for breed, sex and sire group. Adjusted means of the eating quality traits for both hind leg and lumbar region muscles are presented in Table 5.4.

There were significant differences in muscularity between breeds in both carcass regions, as well as between sire groups. The TEX means for muscularity were 17% and 15% greater than those for SBF lambs, in the hind leg and lumbar region respectively. The differences between sire groups were somewhat smaller (approximately 4%) in both regions, which is expected as only a small part of the sire difference (Table 5.1) is inherited and passed onto the lambs. Ewe lambs had slightly but significantly higher values of $HLMI_{CT}$ than ram lambs, whilst the sexes had similar values for $LRMI_{CT}$ ($P > 0.05$).

Among the three fixed effects, breed had the strongest effect on meat eating quality, whereas sex affected only two of these traits and the sire group none. Significant differences were found between breeds for texture, lamb flavour and overall liking for both muscles, and for juiciness in the loin. Meat from SBF lambs was more tender and had stronger lamb flavour than TEX meat. The adjusted means for overall liking indicated that the trained panellists preferred SBF meat.

The effect of muscularity between sire groups was not significant for eating quality of hind leg or lumbar region meat ($P>0.05$). Similar results were found for the comparison between sexes for the hind leg ($P>0.05$). However, the differences between sexes for the adjusted means for abnormal flavour and overall liking were significant ($P \leq 0.05$) for the lumbar region muscle. Meat from ram lambs had a stronger abnormal flavour than that from ewe lambs. On the other hand, the adjusted means for overall liking were higher for meat from ewe than that from ram lambs.

Table 5.3: Adjusted means for hind leg ($HLMI_{CT}$) and lumbar region ($LRMI_{CT}$) muscularity for breed, sex and sire groups, and significance of the effects

EFFECTS		ADJUSTED MEANS	
		$HLMI_{CT}$	$LRMI_{CT}$
BREED	TEX	7.94	0.85
	SBF	6.80	0.74
	Sig	**	**
	s.e.d.	0.08	0.01
SEX	RAM	7.30	0.79
	EWE	7.45	0.80
	Sig	*	ns
	s.e.d.	0.07	0.01
SIRE	HM-sire	7.52	0.81
GROUPS	LM-sire	7.22	0.78
	Sig	**	*
	s.e.d.	0.10	0.01

* $P \leq 0.05$; ** $P < 0.01$; ns $P > 0.05$.
TEX: Texel; SBF: Scottish Blackface; HM: high muscularity; LM: low muscularity
s.e.d.: standard error of the difference

The estimates of regression coefficients of eating quality traits on $HLMI_{CT}$ and $LRMI_{CT}$ are shown in Table 5.5 for SBF and TEX breeds. The associations with the CT muscularity indices were not significant, except for juiciness in the hind leg, in which a significant negative association was estimated (-0.311 ± 0.09; $P<0.01$) in TEX only. Regression coefficient estimates for the lumbar region had larger standard errors that those for the hind leg due to differences in magnitude of the $LRMI_{CT}$ compared to $HLMI_{CT}$. The sizes of the muscularity indices were different by a factor of 10 (Table 5.3).

Table 5.4: Adjusted means of eating quality traits for breed, sex and sire group, and significance of the effects

HIND	BREED[§]				SEX				SIRE GROUP[§]			
LEG [†]	TEX	SBF	s.e.d.[‡]	Sig	RAM	EWE	s.e.d.	Sig	HM-sire	LM-sire	s.e.d.	Sig
Texture	4.33	4.82	0.16	**	4.52	4.63	0.22	ns	4.51	4.64	0.19	ns
Juiciness	4.76	4.86	0.06	ns	4.88	4.74	0.13	ns	4.75	4.88	0.12	ns
Lamb flavour	3.87	4.24	0.11	**	4.11	4.00	0.16	ns	4.02	4.09	0.13	ns
Abnormal flavour	2.98	2.96	0.07	ns	3.02	2.93	0.16	ns	3.01	2.94	0.13	ns
Overall liking	3.83	4.15	0.13	*	3.93	4.04	0.19	ns	3.97	4.00	0.16	ns
LUMBAR	BREED				SEX				SIRE GROUP			
REGION [†]	TEX	SBF	s.e.d.	Sig	RAM	EWE	s.e.d.	Sig	HM-sire	LM-sire	s.e.d.	Sig
Texture	4.59	5.27	0.16	**	4.78	5.08	0.25	ns	5.12	4.74	0.24	ns
Juiciness	4.73	5.01	0.10	*	4.85	4.89	0.15	ns	4.97	4.77	0.15	ns
Lamb flavour	3.93	4.35	0.08	**	4.09	4.19	0.15	ns	4.27	4.01	0.14	ns
Abnormal flavour	2.93	2.85	0.08	ns	3.03	2.75	0.14	*	2.88	2.89	0.13	ns
Overall liking	4.01	4.38	0.08	**	4.00	4.39	0.16	*	4.30	4.09	0.14	ns

* $P \leq 0.05$; ** $P < 0.01$; ns $P > 0.05$.; [‡] s.e.d., standard error of the difference.

[§] TEX: Texel; SBF: Scottish Blackface; HM: high muscularity; LM: low muscularity

[†] Samples of *m. semimembranosus* and *m. longissimus lumborum* were assessed from the hind leg and lumbar region, respectively.

Texture (1 – extremely tough, 8 – extremely tender); juiciness (1 – extremely dry, 8 – extremely juicy); lamb flavour intensity (1 – extremely weak, 8 – extremely strong), abnormal flavour intensity (1 – extremely weak, 8 – extremely strong) and overall liking (hedonic) (1 – dislike very much, 8 – like very much).

Table 5.5: Effect of muscularity of the hind leg ($HLMI_{CT}$) and lumbar region ($LRMI_{CT}$) on the eating quality traits of *m. semimembranosus* and *m. longissimus lumborum*, respectively, by breed

CARCASS REGION	TRAITS	MUSCULARITY: slope regression coefficient (s.e.†)			
		TEX‡		SBF	
HIND LEG	Texture	-0.054	(0.139)	0.204	(0.183)
	Juiciness	-0.311**	(0.090)	0.042	(0.112)
	Lamb flavour	-0.067	(0.120)	-0.078	(0.127)
	Abnormal flavour	-0.003	(0.110)	-0.021	(0.122)
	Overall liking	-0.151	(0.126)	-0.041	(0.153)
LUMBAR REGION	Texture	1.264	(1.375)	0.843	(1.532)
	Juiciness	-0.624	(0.797)	0.454	(0.967)
	Lamb flavour	-0.090	(0.653)	1.207	(0.992)
	Abnormal flavour	0.610	(0.711)	-0.611	(0.887)
	Overall liking	0.211	(0.803)	0.739	(1.059)

** P< 0.01; †s.e., standard error
‡TEX: Texel; SBF: Scottish Blackface

5.4 Discussion

5.4.1 Effect of breed on muscularity and eating quality

The significantly greater muscularity of the TEX lambs, which was calculated in this study for both the hind leg and lumbar region, is in agreement with previous studies that concluded that TEX breed had better muscularity compared with other terminal sire breeds (Hopkins *et al.*, 1997; Jones *et al.*, 2002) as well as maternal, dual purpose and hair breeds (Holloway *et al.*, 1994; Leymaster *et al.*, 2006).

Carson *et al.* (2001a,b) carried out a breed comparison including TEX and SBF in which direct measurements of muscularity were not included, although differences in carcass conformation were investigated. The authors found that crossbred TEXxSBF lambs had higher conformation scores than purebred SBF, when compared at constant weight or at the same fat score. Although the use of differences in conformation scores as an indication of differences in muscularity should be interpreted carefully, and at a similar level of fatness, the results reported by Carson *et al.* (2001a,b) suggest that the TEX-sired lambs had better muscularity.

In terms of meat traits and meat eating quality, the majority of studies in the literature have found small or no significant effects of breed (Ellis *et al.*, 1997; Safari *et al.*, 2001; Arsenos *et al.*, 2002; Johnson, 2003). This has led to the conclusion that, although the genetic potential of the lambs determines, to a certain extent, the quality of the meat they produce, breed is not necessarily the dominant factor, especially when compared with feeding treatments (Sañudo *et al.*, 1998b; Arsenos *et al.*, 2002).

The taste panel analysis in this study showed small but statistically significant differences for most of the sensory traits between the TEX and SBF breeds, in both the hind leg and lumbar region joints. Meat from SBF lambs was more tender than TEX meat. Results from a breed evaluation experiment indicated that TEX meat ranked intermediate for tenderness (Leymaster *et al.*, 2006), although no significant differences were found by Safari *et al.* (2001) and Ellis *et al.* (1997) when comparing TEX to other terminal sire breeds or by Carson *et al.* (2001b) between TEXxSBF and purebred SBF lambs.

Both TEX and SBF had similar and low ratings for abnormal flavour, although lamb flavour was stronger in SBF meat compared to TEX. Overall, meat of SBF lambs was preferred. Although trained panellists performed the evaluation, these results also gave an indication of how these characteristics may be perceived and judged by consumers (Sañudo *et al.*, 1998a,b). However, conclusions from hedonic traits such as overall liking should not be generalised and extrapolated to other markets because the association between flavour and overall acceptability may differ between consumers from different countries, due to regional preferences and culinary habits (Sañudo *et al.*, 1998a).

The differences in eating quality between the breeds may be linked to differences in fat content, especially intramuscular fat (IMF), among other characteristics. Other workers have shown positive effects of fat on eating quality traits in sheep (Dikeman, 1987). Values for IMF percentage in the *m. longissimus lumborum* were 2.14 and 1.33 for SBF and TEX, respectively. This difference in the content of intramuscular fat may be associated with the higher scores for texture, juiciness and flavour in SBF lambs.

5.4.2 Effect of sex on muscularity and eating quality

Consistent differences in some aspects of carcass quality between ram and ewe lambs have been reported in the literature, with carcasses from ram lambs being heavier at the same age

and having lower dressing out percentages and poorer conformation scores, although they also have a lower proportion of fat and higher lean meat yields (Dransfield *et al.*, 1990; Wolf *et al.*, 2001; Johnson 2003; Johnson *et al.*, 2005b). Because of their higher growth rate and leanness, meat from ram lambs may be preferred by consumers who avoid higher levels of fat (Dransfield *et al.*, 1990), with the advantage to producers of achieving target slaughter weights faster. The main concern, however, has been the palatability of the meat produced by ram lambs, in particular tenderness and flavour (Butler-Hogg *et al.*, 1984; Dransfield *et al.*, 1990).

No difference between sexes in muscularity of the lumbar region was found in our study. In the case of the hind leg, there was a small but significant difference. Ewe lambs had higher values for muscularity in this region compared to ram lambs, which is in agreement with Johnson *et al.* (2005b). However, other studies have suggested that sex has no effect on leg muscularity (Purchas and Wilking, 1995; Hopkins, 1996; Hopkins *et al.*, 1997; Wolf *et al.*, 2001) or on the muscularity of the lumbar region (Wolf *et al.*, 2001).

In agreement with other studies, lamb sex had a weak influence on eating quality. The effect of sex was only statistically significant for abnormal flavour and overall liking in the *m. longissimus lumborum*. A more intense abnormal flavour was found in ram lambs in this muscle. A similar trend was observed for the *m. semimembranosus* but the difference was not significant. Although the panellists did not show a clear preference between meat from ram or ewe lambs in the case of the hind leg, they preferred the *m. longissimus* of ewe lambs, probably because of the less intense abnormal flavour.

The higher scores of abnormal flavours in the meat from ram lambs were also identified by Butler-Hogg *et al.* (1984). Similarly, the results obtained by Arsenos *et al.* (2002) did not reveal significant effects of sex on eating quality characteristics except for flavour, where ewe lambs gave more desirable meat than ram lambs especially when heavier carcasses were assessed. Differences in flavour between ewe and ram lambs can also be affected by the slaughter age. More intense abnormal flavours in ram lambs at older ages could be due to a possible male sex effect (Rousset-Akrim *et al.*, 1997).

Our results are in agreement with the view that differences between sexes in palatability traits are not very important (Dransfield *et al.*, 1990; Ellis *et al.*, 1997; Sañudo *et al.*, 1998b; Arsenos *et al.*, 2002), although they also indicate the risk of the presence of abnormal flavours in ram lambs that consumers may dislike.

5.4.3 Muscularity and eating quality

Muscularity indices and measurements of muscle depth are indicators of the shape of the muscle, which is associated with better carcass conformation, higher meat yields and larger muscles that provide more visual appeal from the consumer's point of view (Jones *et al.*, 2002; Jones *et al.*, 2004; Navajas *et al.*, 2007, in Chapter 4).

Estimations of genetic parameters for muscularity assessed in the live animal using CT showed that it is under additive genetic control (Jones *et al.*, 2004), which is in agreement with the differences in the muscularity indices between the progeny of high and low muscularity rams used in this study. *In vivo* muscularity indices have favourable phenotypic and genetic associations with other carcass quality traits that are economically important, such as conformation and fat scores, as well as lean yield and fatness (Jones *et al.*, 2004; Navajas *et al.*, 2007, in Chapter 4). However, knowledge of the relationships with eating quality is critical to predict the consequences on these traits of selecting for improved muscularity.

Differences in muscle development due to various major genes differ in their impact on meat quality, in particular on tenderness. Important concerns arise from the large negative effect of the Callipyge gene on meat quality (Freking *et al.*, 1999). The Callipyge gene, which was identified in a Poll Dorset flock, improves muscularity through increasing muscle development without affecting the bone dimensions (Jackson *et al.*, 1997). The Carwell gene (or Rib-eye muscling gene) is another gene also identified in this breed, which produces an increase of 10% in the *m. longissimus* area but has less severe negative effect on tenderness that can be overcome by normal meat ageing techniques (Jopson *et al.*, 2001).

Beyond the research on the possible pleiotropic effects of major genes for muscle development, there is little information available on the association between muscularity and eating quality from a quantitative point of view. The lack of association between the muscularity of the hind leg and lumbar region and eating quality traits, with the exception of juiciness of *m. semimembranosus* in TEX, is in agreement with the low correlations reported by Johnson *et al.* (2005b) in lambs sired by TEX or TEX/Coopworth. Johnson *et al.* (2005a) did not find significant effects of a QTL for muscling in TEX on meat traits such as pH, colour or shear force. This QTL, as well as others reported by other groups in TEX in similar regions, maps to the likely position of the Myostatin gene. If this is the gene responsible for

improved muscle development, no negative effects on meat quality should be expected (Koohmaraie *et al.*, 2002).

Eating quality of the progeny of HM- and LM-sired lambs was similar for both *m. longissimus* and *m. semimembranosus*, although there were significant differences in the muscularity of both carcass regions, indicating that selection for muscularity would not have an unfavourable effect on these traits. However, these results are in contrast to the adverse effect on eating quality of progeny of Poll Dorset sires with high estimated breeding value for eye muscle depth, which was reported by Hopkins *et al.* (2005). This difference may be partially explained by the use of one sire that was homozygous for the Carwell gene (Hopkins *et al.*, 2005). It is also important to consider that the lack of significant differences in our study between HM and LM sires on the eating quality of their progeny could be due to the fact that the magnitude of the differences in muscularity did not produce large enough changes in eating quality to be detected by the taste panel. In this sense, it would be important to explore further the implications of high muscularity on the intrinsic factors affecting eating quality such as IMF and muscle properties. This information would allow a better understanding of the biological basis and an evaluation of the consequences of larger differences of muscularity on eating quality.

5.5 Conclusions

In conclusion, the small differences between sexes for both muscularity and eating quality found here imply that ram and ewe lambs would be able to supply the market with a final product of similar characteristics, although the incidence of abnormal flavour in ram lambs could become a limitation in the acceptability by consumers. Important differences were quantified for muscularity between the TEX and SBF breeds, but only small but significant differences in term of eating quality were identified between the breeds. These differences indicate complementarity in both carcass and meat eating quality from the use of crossbreeding. In terms of selection within breed, the lack of significant associations between muscularity and eating quality suggest that improvements in muscularity would not have a deleterious effect on eating quality in TEX and SBF breeds.

Chapter 6: Genetic parameters of *in vivo* muscularity indices, body composition and muscle density assessed by computed tomography

6.1 Introduction

Lamb production is an important part of UK agriculture, contributing more than 10% of total livestock output (Defra, 2005). It also makes a very important contribution to maintaining employment and infrastructure in rural communities and helps manage and enhance landscape and biodiversity, especially in less favoured areas. However, for the UK sheep industry to continue as a major producer and exporter of lamb it is essential that its economic viability is improved. To do so, it has to provide carcasses that better meet market requirements, since currently only ca. 55% of UK lambs meet core target specifications (MLC, 2006, unpublished). Improving lamb carcass quality by increasing lean meat output without a corresponding increase in fatness has been the focus of breeding programmes in the UK since the mid 1980's. This has been achieved utilising new technologies such as ultrasound and more recently CT scanning that aim to help breed sheep with more lean and less carcass fat.

CT is a useful breeding tool as it provides very accurate *in vivo* information on sheep body composition is obtained by X-ray computed tomography (CT) (Karamichou *et al.*, 2006b; Macfarlane *et al.*, 2006). CT predictions of carcass muscle (CMW_{CT}) and fat weights (CFW_{CT}) are used in sheep breeding programmes in the UK, with the objective of increasing muscle weight with a minimum change in fat weight. The genetic improvement of other characteristics related to the carcass and meat quality, such as muscularity and eating quality, is now under consideration.

Muscularity is an economically important trait for breeders and meat traders due to its association with conformation, which is part of the carcass classification system in the European countries (CEC, 2002). It was defined by De Boer *et al.* (1974) as the depth of muscle relative to a skeletal dimension. Improved muscularity may improve conformation without increasing fatness. Purchas *et al.* (1991) proposed an index that is consistent with the definition of muscularity, in which the weight of the muscle and the length of the bone are combined. This approach cannot be used in live animals, which restricts its utility to improve muscularity genetically. However, Jones *et al.* (2004) described an accurate muscularity index for the whole carcass based on this approach, using CMW_{CT} and the length of the spine both measured by CT. Two other assessments of muscularity of the hind leg (HL) and lumbar region (LR) were proposed by Jones *et al.* (2004), which are the result of combining muscle depth and width in the HL, and muscle depth and spine length for the LR. Recent developments on the direct assessment of muscle mass in the HL and LR based on three-

dimensional (3D) information given by spiral CT scans (SCTS) (Navajas *et al.*, 2006a, in Chapter 3), in combination with new measurements of skeletal dimensions, enabled the development of new muscularity indices for these regions using Purchas's approach (Navajas *et al.*, 2007, in Chapter 4). These measurements have shown to be highly accurate in Texel (TEX) and Scottish Blackface (SBF), two divergent breeds relevant in the UK lamb industry.

Meat quality traits are also of high relevance because of increasing consumers and retailers expectations. Because meat quality can only be assessed after slaughter, *in vivo* ultrasound assessments of intramuscular fat (IMF) have been used in beef cattle and pigs as a predictor of meat quality (Lambe and Simm, 2004), although this image technology has not been applied in sheep. IMF is a meat trait of interest due to its positive association with meat eating quality. Higher contents of IMF are linked to higher tenderness, flavour and juiciness, and therefore it has a positive general effect on palatability (Savell and Cross, 1988). Values of 2 to 3% of IMF, as suggested by Savell and Cross (1988), would indicate the possibility of achieving a palatable and healthy product. Karamichou *et al.* (2006b) showed that muscle density measured *in vivo* by CT is genetically correlated to lamb eating quality and IMF content in SBF lambs.

The inclusion of these new traits into breeding programmes requires knowledge of their heritabilities and of the genetic relationships among all characteristics of interest. This study presents estimates of genetic parameters for CT muscle density, carcass composition and CT measurements of muscularity of carcasses and joints in TEX and SBF lambs. Some of these results were described by Navajas *et al.* (2006b).

6.2 Materials and methods

6.2.1 Animals

Data were recorded on 228 TEX and 238 SBF female and entire male lambs that were born in 2003 and 2004 from purebred flocks at a SAC experimental farm. The SBF and TEX lambs were from 11 and 10 sires, and an average of 16 and 17 dams per sire, respectively. The pedigree file used contained 1064 records (TEX=506; SBF=558), including the sires, dams and paternal grandparents of all lambs. Information was also included on maternal grandparents for 190 TEX and 83 SBF lambs, paternal great-grandparents for 103 TEX and 53 SBF lambs and maternal great-grandparents of 66 TEX and 76 SBF lambs.

Lambs grazed in single sex, mixed-breed groups from weaning to finishing, which was defined as typical commercial slaughter point in the UK (condition score ≥ 3 and live weight (LW) ≥ 35 kg). Each batch of finished lambs was of mixed breed and sex. Half of the lambs in each finishing batch (balanced for breed and sex) were slaughtered at finishing. The other half of the lambs of each batch were slaughtered 30 days later, the withdrawal period for the sedative used as a pre-requisite for CT scanning, to allow taste panel analyses also performed in this study (data not presented). More information is given in Chapter 2.

6.2.2 Carcass composition and muscularity measurements by CT

Lambs were CT scanned at finishing. The CT protocol included: (1) longitudinal scan (topogram); (2) cross-sectional scans positioned at the end of the ischium bone (ISC), the 5th lumbar vertebra (LV5) and the 8th thoracic vertebra (TV8); and (3) SCTS in which contiguous cross-sectional scans of constant thickness were collected from the cranial third of the tibia to the 4th cervical vertebra.

Carcass muscle weight (CMW_{CT}, kg) was predicted from the muscle areas in ISC, LV5 and TV8 scans plus LW (TEX, Macfarlane *et al.*, 2006; SBF Karamichou *et al.*, 2006b). The muscle volume in HL ($HLMV_{CT}$, cm^3) and LR ($LRMV_{CT}$, cm^3) were obtained from the SCTS (Navajas *et al.*, 2006a, in Chapter 3). The spine length (SPL_{CT}, cm) was measured on the topogram from the first rib to the cranial side of the pelvis (Jones *et al.*, 2002). A new method to assess femur length was developed using the 3D reconstruction of SCTS (Navajas *et al.*, 2007, in Chapter 4). The femur length was measured in both legs and them averaged (FL_{CT}, cm).

Two CT measurements of muscularity were computed as <u>combinations of linear dimensions</u>: (1) *Hind leg shape* (HLS_{CT}): depth (D_{ISC}) and width (W_{ISC}) of the hind leg muscle, and the thickness of the popliteal fat depot (P_{ISC}) were measured in the ISC scan. The ratio of D_{ISC} minus P_{ISC} to W_{ISC} was multiplied by 10 and averaged over both legs (Jones *et al.*, 2002); and (2) *LTL muscularity index* ($LTLMI_{CT}$): the ratio between the depth of the muscle in the LV5 and SPL_{CT} was also computed as a measure of the LR muscularity (Jones *et al.*, 2002).

Muscularity indices were also calculated as <u>combinations of muscle mass and skeletal dimensions</u>: (1) *Carcass muscularity index* (CMI_{CT}) = ($\sqrt{(CMW_{CT}/SPL_{CT}^3)}$) x 10; (2) *HL muscularity index* ($HLMI_{CT}$) = ($\sqrt{(HLMV_{CT}/FL_{CT}^3)}$) x 10; and (3) *LR muscularity index* ($LRMI_{CT}$) = ($\sqrt{(LRMV_{CT}/SPL_{CT}^3)}$) x 10.

6.2.3 CT muscle density and intramuscular fat

The average CT muscle density in the cross-sectional reference scans taken in the hind leg region (ISCMD) and lumbar region (LV5MD) were also included in this study. The average muscle density is given by the average CT values of the pixels that correspond to muscle (Wegener, 1993).

The content of IMF in the *m. gracilis* (hind leg) and *m. longissimus lumborum* (lumbar region) were also determined by chemical analysis (IMFGR and IMFLD, respectively). IMFGR was estimated as the total amount of all phospholipid and neutral lipid fatty acids. The day after slaughter, the *m. gracilis* was dissected from the left hind leg and half the muscle was used for fatty acid analysis. After thawing, any small amounts of adhering adipose or connective tissue were removed and the muscle was homogenized in a food processor. The fatty acids were extracted by direct saponification, methylated and analysed by gas-liquid chromatography, following the method of Doran *et al.* (2006).

A frozen cross-sectional slice of the *m. longissimus lumborum* was removed from its cranial end, vacuum packed and frozen. After thawing, each sample was blended to a fine paste using a laboratory blender. Sub-samples (25 mg) were weighed into pre-dried and weighed plastic pots, frozen, and freeze dried (72 hr) using an Edwards Modulyo Unit (BOC Edwards, Wivelsfield Green, West Sussex, UK). IMFLD was extracted from each of the dried and crushed samples using petroleum ether (B.P. 40-60 °C) as the solvent in a modified Soxhlet extraction using an automatic Gerhardt Soxtherm 2000 unit (Gerhardt Gmbh, Koningswinter, DE).

6.2.4 Statistical analyses

Variance components were estimated by univariate and bivariate analysis, within breed, by restricted maximum likelihood in ASREML (Gilmour *et al.*, 2002). Mixed animal models were fitted, which included: fixed effects of birth year, sex, litter size reared (single or multiple, and artificially reared only in TEX) and dam age (2, 3, 4 and 5+ years) and age at finishing as a linear co-variate, along with a random animal effect. For IMFLD and IMFGR, slaughter group (slaughtered after scanning or 30 days later) was also fitted as fixed effect.

6.3 Results and discussion

Means and coefficients of variation for the traits included in this study are shown in Table

6.1, and the estimates of genetic parameters are presented in Tables 6.2 to 6.4.

Table 6.1: Means and coefficients of variation (CV) for CT traits, intramuscular fat content and age at finishing

Traits[‡]	TEX[§]		SBF	
	Mean	CV (%)	Mean	CV (%)
Age (days)	134.2	18.2	144.4	16.4
LW (kg)	37.08	10.0	34.43	9.5
CFW_{CT} (kg)	2.51	37.1	3.18	30.8
CMW_{CT} (kg)	11.24	12.8	8.02	12.5
$LTLMI_{CT}$	0.65	10.8	0.54	14.8
$LRMI_{CT}$	0.86	8.1	0.73	9.6
HLS_{CT}	5.79	11.7	4.27	15.9
$HLMI_{CT}$	8.08	6.1	6.81	7.2
CMI_{CT}	3.48	6.9	2.81	6.0
IMFLD (%)	1.60	49.8	2.28	35.9
IMFGR (mg/100g muscle)	1865	43.7	2374	36.1
ISCMD (grey scale units)	49.32	3.6	46.11	3.5
LV5MD (grey scale units)	48.38	5.4	44.67	4.7

[‡] CFW_{CT}, carcass fat weight predicted by CT; CMW_{CT}, carcass muscle weight predicted by CT; $LTLMI_{CT}$, LTL muscularity index; $LRMI_{CT}$, Lumbar region muscularity index; HLS_{CT}, hind leg shape; $HLMI_{CT}$, hind leg muscularity index; CMI_{CT}, carcass muscularity index; IMFLD, content of intramuscular fat in *m. longissimus lumborum*; IMFGR, content of intramuscular fat in *m. gracilis*; ISCMD, muscle density measured in reference scan at ischuim; LV5MD, muscle density measured in reference scan at the 5th lumbar vertebra. [§] TEX, Texels; SBF, Scottish Blackface.

6.3.1 CT prediction of tissue weights and muscularity indices

Estimates of heritabilities of CT prediction of tissue weights and muscularity indices for both breeds are in Table 6.2. Phenotypic and genetic correlations between muscularity indices are presented in Table 6.3.

There are only few studies on genetic parameters for carcass traits predicted by CT in sheep (terminal sire breeds, Jones *et al.*, 2004, Kvame, 2005, Macfarlane, 2006; SBF lambs, Conington *et al.*, 2006b, Karamichou *et al.*, 2006b). The heritabilities estimated in this study for CMW_{CT} in TEX and SBF were similar to most previous estimates (0.43 - 0.49). The heritability estimates for CFW_{CT} were larger than the values previously reported in both breeds (terminal sire breeds: 0.18 - 0.41: Jones *et al.*, 2004, Kvame, 2005, Macfarlane, 2006;

SBF: 0.35 - 0.60: Conington *et al.*, 2006b, Karamichou *et al.*, 2006b).

The estimates of heritability of the muscularity indices tended to be similar or lower in TEX than in SBF, except for $HLMI_{CT}$, which had the highest heritability. The estimates for $LTLMI_{CT}$ and CMI_{CT} in TEX were similar to the values of 0.42 and 0.48 obtained by Jones *et al.* (2004). However, the heritability for HLS_{CT} was larger than the estimate reported for TEX (0.29) but closer to those in other terminal sire breeds (~0.38). The estimate for HLS_{CT} in SBF seems to be higher than the 0.44 reported by Conington *et al.* (2006b).

Table 6.2: Heritabilities ($h^{2)}$ for muscularity indices and CT predictions of carcass muscle and fat weights

CT traits‡	TEX§		SBF	
	h^2	s.e.†	h^2	s.e.
LW	0.15	(0.13)	0.25	(0.15)
CFW_{CT}	0.53	(0.23)	0.70	(0.21)
CMW_{CT}	0.47	(0.20)	0.43	(0.19)
$LTLMI_{CT}$	0.38	(0.20)	0.59	(0.19)
$LRMI_{CT}$	0.38	(0.23)	0.62	(0.19)
HLS_{CT}	0.44	(0.19)	0.66	(0.20)
$HLMI_{CT}$	0.92	(0.19)	0.78	(0.18)
CMI_{CT}	0.47	(0.20)	0.42	(0.19)

‡ Abbreviations are defined in Table 6.1. §TEX, Texels; SBF, Scottish Blackface. †s.e.: standard errors

The genetic correlations were positive and moderate to high (Table 6.3), except those between HLS_{CT} and all indices other than $HLMI_{CT}$ in TEX, which were positive, but not significantly different from zero. This agrees with the results of Jones *et al.* (2004).

When comparing the results between the new indices for HL and LR, and HLS_{CT} and $LTLMI_{CT}$, the conclusions differ between regions and breeds. The muscularity indices for LR were strongly correlated and had similar estimates of heritability in each breed (TEX, 0.38; SBF, ~0.60), suggesting that the genetic response for LR muscularity would be similar from using $LTLMI_{CT}$ or $LRMI_{CT}$ in both breeds. These indices had a similar moderate accuracy as measurements of LR muscularity assessed post-slaughter (r~0.50, Navajas *et al.*, 2007, in Chapter 4). Because muscle shape along the spine is more homogenous, the muscle

depth used in $LTLMI_{CT}$ seems to provide a good measure of shape. However, indices for the HL were strongly correlated in both breeds, but the estimates of heritability were higher for $HLMI_{CT}$, particularly in TEX. This difference may indicate advantages of using $HLMI_{CT}$ to improve the muscularity in the HL. $HLMI_{CT}$ was much more accurate than HLS_{CT} (r= 0.90 *vs* 0.50, Navajas *et al.*, 2007, in Chapter 4), but it is more time consuming to measure. The greater complexity of the HL shape may be one of the reasons explaining the differences between HLS_{CT} assessed at one anatomical position and the muscularity of the joint, which is more pronounced in TEX than SBF.

Table 6.3: Phenotypic and genetic correlations (above and below the diagonal, respectively) for CT muscularity indices. Standard errors are in brackets

Breed	Index‡	$LTLMI_{CT}$	$LRMI_{CT}$	HLS_{CT}	$HLMI_{CT}$	CMI_{CT}
TEX§	$LTLMI_{CT}$		0.66	0.28	0.45	0.62
			(0.04)	(0.07)	(0.07)	(0.05)
	$LRMI_{CT}$	NC†		0.26	0.40	0.75
		--		(0.07)	(0.07)	(0.04)
	HLS_{CT}	0.27	0.21		0.53	0.25
		(0.35)	(0.38)		(0.06)	(0.07)
	$HLMI_{CT}$	0.67	0.52	0.86		0.38
		(0.17)	(0.21)	(0.21)		(0.07)
	CMI_{CT}	0.85	0.60	0.42	0.56	
		(0.13)	(0.21)	(0.35)	(0.20)	
SBF	$LTLMI_{CT}$		0.75	0.43	0.50	0.63
			(0.03)	(0.07)	(0.06)	(0.05)
	$LRMI_{CT}$	0.87		0.38	0.50	0.79
		(0.09)		(0.07)	(0.06)	(0.03)
	HLS_{CT}	0.63	0.77		0.54	0.30
		(0.17)	(0.15)		(0.06)	(0.07)
	$HLMI_{CT}$	0.52	0.62	0.83		0.37
		(0.19)	(0.17)	(0.11)		(0.07)
	CMI_{CT}	0.87	0.94	0.68	0.65	
		(0.13)	(0.06)	(0.20)	(0.20)	

†NC: no convergence. ‡ Abbreviations are defined in Table 6.1. §TEX, Texels; SBF, Scottish Blackface

In SBF, the genetic associations with CMI_{CT} were very strong for LR indices and somewhat weaker for the HL. In TEX, the associations differed between indices. The correlations were around 0.60 with the new indices, whilst it was very high with $LTLMI_{CT}$ and not significantly different from zero with HLS_{CT}. Similarly, the association between LR and HL in TEX differs between indices. LR muscularity indices were not significantly correlated with HLS_{CT}, but had coefficients around 0.60 with $HLMI_{CT}$. Both HL indices in SBF were correlated with LR indices, with values ranging between 0.52 and 0.77. The differences between breeds imply that, if improving muscularity of more that one region of the carcass is the aim, different indices (or combinations of indices) would optimise the genetic response in each breed.

Although the estimates agree with previous reports, they should be interpreted with caution because of their high standard errors, due to the low number of records and shallow pedigree available. A simple animal model was preferred because of this. Although the exclusion of maternal genetic and environmental effects may have altered the estimates, recent studies of larger datasets indicated that they had little effect on CT traits (Macfarlane, 2006).

6.3.2 CT muscle density and intramuscular fat

CT scanning makes use of the different rates at which the major tissues in the body attenuate X-rays depending on the densities of the tissues. CT muscle density is the average pixel value for this tissue and is related to real density of the tissue, which depends on the chemical composition and water content. One of the components determining muscle density is IMF, which is less dense than the muscle fibre fraction. Therefore, a higher concentration of IMF will reduce muscle density.

High heritabilities were estimated for CT muscle density (SBF = 0.56; TEX = 0.79) and IMF (SBF = 0.82; TEX = 0.80) in the lumbar region for both breeds, with standard errors around 0.2. Strong negative phenotypic correlations were found between these traits in both breeds (r_P: -0.63 SBF; -0.72, TEX). The genetic correlation in SBF was -0.90 (standard error = 0.10) but it could not be estimated in TEX. Heritability of ISCMD could not be estimated in TEX (no convergence) and was not significantly different from zero for SBF. Therefore, investigations into relationships with IMF were concentrated on the lumbar region.

The estimated heritability of LV5MD indicates is under moderate to high genetic control. Values of 0.35 to 0.81 were reported by Karamichou *et al.* (2006b) for CT muscle density in

SBF measured in different anatomical positions.

Table 6.4: Phenotypic and genetic correlations (standard errors) for CT muscularity indices and predicted carcass composition with CT muscle density (LV5MD) in SBF and TEX lambs.

CT Traits‡	TEX§		SBF	
	r_G	r_P	r_G	r_P
LW	0.13	- 0.18	0.24	0.04
	(0.42)	(0.07)	(0.38)	(0.08)
CFW_{CT}	- 0.69	- 0.56	- 0.76	- 0.44
	(0.17)	(0.06)	(0.17)	(0.06)
CMW_{CT}	0.66	0.10	0.28	0.21
	(0.12)	(0.10)	(0.31)	(0.08)
$LTLMI_{CT}$	NC†	0.10	0.08	0.15
	--	(0.07)	(0.29)	(0.08)
$LRMI_{CT}$	0.64	0.06	0.05	0.20
	(0.38)	(0.07)	(0.30)	(0.08)
HLS_{CT}	0.34	0.15	0.18	0.12
	(0.31)	(0.08)	(0.28)	(0.08)
$HLMI_{CT}$	0.20	0.09	-0.20	0.01
	(0.21)	(0.09)	(0.26)	(0.08)
CMI_{CT}	0.57	0.12	0.21	0.14
	(0.27)	(0.08)	(0.33)	(0.08)

†NC: no convergence. ‡Abbreviations are defined in Table 6.1. §TEX, Texels; SBF, Scottish Blackface

Low phenotypic correlations (in most cases not significantly different from zero) were estimated between LV5MD and the muscularity indices, LW or CMW_{CT} (Table 6.4). In both breeds the phenotypic correlation with CFW_{CT} was around -0.5. Little association was found between muscularity indices and CT muscle density, implying that improved muscularity would not have a negative effect on CT muscle density. Large standard errors for genetic correlations with LV5MD limit their interpretation, but some breed differences were observed, with a trend for higher (more positive) correlations with CMW_{CT} and muscularity indices in TEX, compared to SBF lambs. A strong negative genetic correlation was estimated between LV5MD and CFW_{CT} in both breeds. This would be expected because of

the association between CT muscle density and IMF (negative) and that between carcass fatness and IMF (positive).

The genetic relationships between CT muscle density and the muscularity indices did not indicate any antagonism nor did the relationships with CT muscle weight. However, there are strong unfavourable associations with CFW_{CT} in both breeds, probably due to the unfavourable association between IMF and carcass fat (Wood, 1990; Thompson and Ball, 1997). This reinforces the relevance of including this *in vivo* measurement of muscle density in order to ensure that the levels of IMF remain above the critical levels for consumers' satisfaction.

6.4 Conclusions

The heritabilities of muscularity indices assessed using CT were moderate to high in TEX and SBF breeds. Although the values should be used with caution due to the large standard errors, these estimates show that there is scope for genetic improvement of muscularity of the whole carcass and of regions where high priced cuts are located, HL and LR, in both breeds. The improvement of muscularity in different regions of the carcass may require the utilisation of different CT indices as selection criteria in these breeds.

Muscle density measured by CT is a promising technique for selecting live animals for IMF content, and hence improved meat eating quality. Although selection for CT muscle density would be neutral or have a minimal effect on carcass muscularity and muscle content, there is an unfavourable relationship between carcass fat and CT muscle density.

The information available indicates that CT can be used to genetically improve these traits, although estimates of genetic parameters need to be confirmed in larger data sets.

Chapter 7: Breeding for carcass and meat quality using a two-stage selection strategy in sheep

7.1 Introduction

Intramuscular fat (IMF) is a meat trait of interest due to its positive association with meat eating quality. Higher contents of IMF are linked to higher tenderness, flavour and juiciness, and therefore it has a positive general effect on palatability (Savell and Cross, 1988). Although IMF plays a key role in terms of eating quality, the magnitude of the association has been controversial. Contents of 2 to 3% of ether extractable fat (measure of IMF) have been identified as the minimum levels to achieve acceptable consumer satisfaction for grilled red meat cuts including beef and lamb meat (Dikeman, 1987; Savell and Cross, 1988).

Because it is impossible to measure meat quality traits directly in the live animal, IMF has been used in beef cattle and pork as a predictor of meat quality (Lambe and Simm, 2004). Breeding values for IMF measured by ultrasound in beef cattle are available in USA and Australia (Hassen *et al.*, 2003). We know of no studies on the utilisation of ultrasound scanning in sheep to assess IMF.

X-ray computed tomography (CT) is another imaging technology used in sheep breeding programmes, which provides very accurate predictions of body composition (Macfarlane *et al.*, 2006) and muscularity (Jones *et al.*, 2002; Navajas *et al.*, 2007, in Chapter 4). CT scanning makes use of the different rates at which the major tissues in the body attenuate X-rays, which depends on the real densities of the tissues (Imaginis, 2005). CT muscle density is related to the attenuation values of this tissue, expressed as the average pixel value, and depends on the tissue chemical composition and water content. One of the components determining muscle density is the content of IMF, which is less dense than the muscle fibre fraction. Therefore, a higher concentration of IMF will reduce the CT muscle density (Goodpaster *et al.*, 2000).

Karamichou *et al.* (2006b) reported strong genetic associations between CT muscle density and eating quality traits in Scottish Blackface lambs. Flavour, juiciness and overall palatability were negatively correlated with CT muscle density, with estimates of genetic correlations that ranged between –0.70 and –0.80, although the estimated genetic correlation with toughness was close to zero.

CT scanning is a very accurate technique but also expensive. A cost-effective use of this tool can be achieved as part of two-stage selection programmes (Jopson *et al.*, 1997, 2004;

Young *et al.*, 2001a), such as those proposed for the terminal sire breeds in the UK. The breeding objective in most terminal sire breeds is to increase carcass muscle weight (CMW) with minimum increase in carcass fat weight (CFW). Selection has been based on the lean index proposed by Simm and Dingwall (1989), which combines information on live weight, ultrasound muscle depth and fat depth, and, more recently, muscle and fat weight predicted by CT. The relative economic values for the CMW and CFW in the lean growth index were +3 and -1, respectively (Simm and Dingwall, 1989). Recently some breeds in the UK have adopted a slightly modified index which constrains the index scores calculated for sheep with extremely low fat depths (Nieuwhof, 2004).

The objectives of this study were to:

(i) quantify the expected correlated response in IMF in two-stage selection programmes for carcass quality and the effect of different economic values for IMF on the genetic improvement of CMW and CFW;

(ii) examine the impact of including a prediction of IMF using CT on the expected responses in IMF and CMW and CFW assuming different economic values for IMF; and

(iii) evaluate the economic efficiency of including a CT predictor of IMF in a two-stage selection programme.

7.2 Materials and methods

7.2.1 Breeding goal and economic values

The aggregate breeding goal (H) is the combination of the relevant traits to be improved. It can be formulated as $H = a'Y$, where a is the vector of economic values and Y is the vector of genetic values of traits in the breeding goal.

The traits included in the breeding goal were CMW and CFW, which are in the current breeding goal of terminal sire breeds in the UK. IMF was added as the meat quality trait related to eating quality attributes.

The economic values of CFW and CMW used in this study were £ 4.0/kg and £–1.3/kg, respectively, as derived by Young *et al.* (2001a) as benchmark figures. These values are in agreement with the ratio of economic values of 3:-1 that optimises the genetic responses in these traits (Simm and Dingwall, 1989). In this study, H can then be expressed as:

$$H = +4 \times LEAN - 1.3 \times FAT + a_{IMF} \times IMF$$

with a_{IMF} being the economic value of IMF. The values a_{IMF} that were investigated were £1.30, £1.95, £3.90, £5.20 and £6.50. They correspond to economic values of IMF relative to CFW (-1.0) of 1.00, 1.50, 3.00, 4.00, and 5.00, respectively, which were chosen arbitrarily to cover a wide range of relative economic weights. IMF was also included in the objective, with an economic value of zero, in order to quantify the correlated response in IMF due to the current criteria used in selection.

7.2.2 Characteristic measured in the live animals and selection indices

The aggregate breeding goal is improved by selection on an index (I) of criteria traits (X). The selection index is equivalent to $I = b'X$, with b being the vector of the weighting factors for each criterion in the index that maximises the correlation between the breeding goal and the selection index (r_{IH}).

The criteria or characteristics measured in the animal were: fat (UFD) and muscle depth (UMD) measured by ultrasound, live weight at the time of the ultrasound scanning (SLW), weight of fat (CFW_{CT}) and muscle (CMW_{CT}) in the carcass predicted from CT measurements, and muscle density measured by CT (MD_{CT}). UFD and UMD are predictors of body composition, which are measured on lambs at the 3rd lumbar vertebra. CFW_{CT} and CMW_{CT} are predicted weights of these tissues using the area of fat and muscle, respectively, from CT scans at specific anatomical locations (Macfarlane *et al.*, 2006). MD_{CT} is given by the average CT value of those pixels that correspond to muscle in the CT images. The CT values quantify the amount of absorption of the X-rays that depends on the actual density of the tissue (Wegener, 1993). This is related to its chemical composition, and in particular to the content of IMF (Goodpaster *et al.* 2000; Karamichou *et al.*, 2006b).

Two selection indices were calculated:

Index 1 (I1): all animals have only information from ultrasound scanning (SLW, UFD and UMD);

Index 2 (I2): all animals are ultrasound and CT scanned. Therefore, they have information on all six characteristics measured (SLW, UFD, UMD, CMW_{CT}, CFW_{CT} and MD_{CT}).

The values of b for I1 and I2 were obtained by solving the equation:

$b = P^{-1}Ga$

where P is the matrix of phenotypic (co)variances between the criteria traits in X and G is the matrix of genetic (co)variances between the criteria traits and those in those in the breeding goal.

The accuracy of the index (r_{IH}), the genetic response in economic units from one round of selection on the index per standardised selection differential (σ_I), and the correlated genetic response in each objective trait from one round of selection on the index per standardised selection differential (CR_m), were calculated as follows:

$$r_{IH} = \sqrt{\frac{\sigma_I^2}{\sigma_H^2}} \qquad \sigma_I = \sqrt{\sigma_I^2} \qquad CR_m = \frac{b'G_m}{\sigma_I}$$

where:

σ_I^2 is the variance of index with $\sigma_I^2 = b'Pb$;

σ_H^2 is the variance of H with $\sigma_H^2 = a'Ca$, where C is the matrix of genetic (co)variances between the objective traits;

G_m is the mth column in the matrix G, which corresponds to trait m.

7.2.3 Genetic and phenotypic parameters

The phenotypic and genetic parameters for the traits in the breeding goal and indices are presented in Table 7.1.

Multiple sources of data were used. The phenotypic and genetic parameter for traits currently being used in the two-stage breeding programme were the average of those estimated by Macfarlane (2006) for the Suffolk and Texel sire reference schemes. It was assumed that CMW_{CT} and CFW_{CT} were perfect predictors of CMW and CFW.

The parameters for IMF were summarised from the literature. Because of the lack of information on this trait in sheep, estimates of phenotypic and genetic parameters in beef cattle were reviewed, including measurements in the carcass and in live animals using ultrasound. The use of ultrasound to assess IMF in cattle, which is quite common practice in the United States and Australia, was the only *in vivo* measurement currently in use for which information could be found. Information from studies on pigs was not included because the distribution of fat among depots in this species is different from that in ruminants (Kempster *et al.*, 1982).

Table 7.1: Parameters assumed for index calculations. Heritabilities are on the diagonal, phenotypic correlations are above and genetic below the diagonal

	SLW	UMD	UFD	CMW_{CT}	CFW_{CT}	MD_{CT}	IMF
V_G†	7.93	1.75	0.38	1.10	0.69	1.65	0.20
SLW	0.24	0.56	0.46	0.81	0.74	-0.10	0.15
UMD	0.60	0.25	0.31	0.50	0.40	0.05	-0.05
UFD	0.52	0.29	0.29	0.25	0.57	-0.15	0.20
CMW_{CT}	0.82	0.49	0.09	0.43	0.60	0.05	-0.10
CFW_{CT}	0.59	0.29	0.65	0.41	0.39	-0.35	0.40
MD_{CT}	-0.20	0.05	-0.20	0.05	-0.40	0.30	-0.60
IMF	0.25	-0.05	0.35	-0.05	0.60	-0.75	0.40

† V_G= Genetic variances

In the case of MD_{CT}, the estimates of the heritability and genetic and phenotypic correlations were defined based on Karamichou *et al.* (2006b). The value of heritability of 0.30 was closer to the lowest estimates of this parameter for MD_{CT}, because a more conservative approach was preferred. The phenotypic correlation between MD_{CT} and IMF corresponds to an R^2 of 0.36, which is also close to the lowest estimates reported by Young *et al.* (2001a) and Karamichou *et al.* (2006b), which did not include other fat depots as predictors. The magnitude of the genetic correlation between MD_{CT} and IMF used in this study is close to the values reported by Karamichou *et al.* (2006b) and those reported between chemically-estimated IMF and its prediction by ultrasound in beef cattle. The values of the other genetic correlations with MD_{CT} followed the same trend as IMF but were slightly lower. The genetic and phenotypic (co)variance matrices were positive definite.

7.2.4 Genetic gains of one- and two-stage strategies

The overall genetic gain in economic units per generation (ΔG) is given by the contribution of rams and ewes as shown in the following equation:

$$\Delta G = \Delta G_R + \Delta G_E$$

The genetic response of each sex is calculated as:

$$\Delta G_k = 0.5\, i_k\, \sigma_I$$

where k refers to either rams or ewes, i_k is the selection intensity applied in each sex and σ_I

is the standard deviation of the selection index. Annual genetic gains were calculated by dividing ΔG by the average generation interval. The generation intervals for ewes and rams were 3.5 and 2 years, respectively.

Similarly, the genetic gain per generation for the traits in the breeding objective was calculated by replacing σ_I by CR_m.

7.2.4.1 Contribution of ewes

It was assumed that the second stage of selection was only applied to males. Therefore, the contribution of the ewes in both one- and two-stage strategies was calculated in the same way. The proportion of ewes selected as replacements using I1, which is based on ultrasound measurements, was 0.60. The selection intensity was then calculated assuming the truncation of a normal univariate distribution (Falconer and Mackay, 1996).

7.2.4.2 Contribution of the rams

The calculation of the contribution of the males in a one-stage strategy is similar to the approach used for ewes. It was assumed that the best 5% of rams based on I1 or I2 were selected.

In the case of the two-stage strategy, selection intensities were not calculated assuming the truncation of a normal distribution, since the distribution is no longer normal because it has already been truncated by the first round of selection. Selection intensities were calculated based on the approach described by Jopson *et al.* (2004), in which the selection intensities are adjusted after the second stage of selection, to account for the inefficiency of the fact that some good candidates that might have otherwise been selected with full information will be eliminated in the first round of selection, because of lower accuracy of the stage 1 index.

This can be implemented in Mathcad, using a standard bivariate normal (SBN) distribution, which accounts for the prior selection at stage one.

The form of the standard bivariate normal is:

$$SBN(x, y, \rho) = \frac{1}{2\pi\sqrt{1-\rho^2}} e^{\frac{-1}{2(1-\rho^2)}(x^2+y^2-2\rho \cdot x \cdot y)}$$

where:

x is the stage one index

y is the index including the traits used in stage one and two, and

ρ is the correlation between the two indexes (Jopson *et al.*, 2004).

For each set of economic values, the correlation between I1 and I2 (r_{I1-I2}), ρ, was calculated as the ratio of their respective correlations with the aggregate breeding value (r_{I1-H} and r_{I2-H}), since I2 incorporated all traits that were included in I1 and the breeding objective remains the same (Wade and James, 1996):

$$r_{I1-I2} = \frac{r_{I1-H}}{r_{I2-H}}$$

The selection intensity (i_{2S}) of the animals after selection at stage two was:

$$i_{2S} = \frac{\int_{tx}^{\infty}\int_{ty}^{\infty} SBN(x,y,\rho)\cdot x\,dy\,dx}{propn_y}$$

where $propn_y$ is the proportion of animals selected after the second stage of selection (relative to the number of candidates available prior to stage 1 selection) and tx and ty are truncation points of the first and second round of selection respectively (Jopson *et al.*, 2004).

It is necessary to derive threshold values tx and ty which result in the following equalities:

$$\int_{tx}^{\infty}\int_{-\infty}^{\infty} SBN(x,y,\rho)\,dy\,dx = \int_{tx}^{\infty} N(x)\,dx = propn_x$$

$$\int_{tx}^{\infty}\int_{ty}^{\infty} SBN(x,y,\rho)\,dy\,dx = propn_y$$

where $propn_x$ is the proportion of animals selected after stage 1, and N(x) denotes the frequency distribution probability for a standard normal variate x.

In this study, $propn_y$ was 0.05. For the first round of selection, 18 different proportions of candidates selected to go forward for CT scanning ($propn_x$= 0.10 to 0.95) were considered.

The response to two-stage selection is therefore taken as:

$\Delta G = \sigma_{I2} i_{2S}$

where σ_{I2} is the standard deviation of the index available for stage 2 selection with no

downward adjustment to account for stage 1 selection. It should be noted that the Jopson method does not require direct specification of the standard deviation of the stage 1 index, rather, it just requires specification of the genetic correlation between the stage 1 index, and the stage 2 index. The Jopson method also requires proportions of candidates selected after stage 1 and stage 2 selections, as well as the standard deviation of the selection index values for candidates, assuming that selection criteria have been recorded on all candidates (I2) irrespective of whether or not they are selected at stage 1.

7.2.5 Economic evaluation of two- stage programme

The economic evaluation of the two-stage selection strategy was based on the marginal net discounted returns over 20 years ($MNDR$). As some of the economic values were arbitrary, the main value of this economic evaluation is in identifying the key factors affecting economic returns, rather that in estimating expected returns precisely. Selection using a two-stage selection strategy was compared with one-stage selection on ultrasound and live weight alone. This was obtained as:

$$MNDR = ((\Delta G_{2S} - \Delta G_{1S}) \cdot (NR_{sold} \cdot DP)) - (NR_{scan} \cdot DC)$$

where ΔG_{1S} is the annual genetic response in economic units from selection on stage one measurements alone in which ram and ewe lambs are selected based on I1;

ΔG_{2S} is the annual genetic response in economic units from selection using a two-stage selection strategy, where selection of ewe lambs is based on I1 and a proportion of the best ram lambs based on I1 are CT scanned. The proportions evaluated ranged between 0.10 and 0.95;

NR_{sold} is the number of rams sold per year;

DP is the discounted contribution per selected ram in the commercial tier;

NR_{scan} is the number of rams CT scanned per year; and,

DC is the cumulative discounted cost of CT scanning one ram per year over 18 years.

The cost of ultrasound scanning was not included since it occurs in both one-stage and two-stage selection strategies.

7.2.6 Number of rams CT scanned and rams sold

The number of rams born in the sire reference scheme was calculated assuming a total number of ewes in the breeding flock of 6000 and a ratio of 1.25 lambs at recording per ewe. These parameters are representative of the Texel and Suffolk reference schemes according to Young *et al.* (2001a). It was assumed that 75% of the ram lambs are sold for crossing.

Discounted expressions of costs and returns were calculated to take into account the fact that both cost and economic benefit at time t are correspondingly more valuable than cost and benefits at time t+1. The actual cost (AC) of CT scanning per ram assumed was £65. The discounted cost per ram was £24.21, considering an inflation free discount rate (d) of 5%, a time horizon for appraisal of investment (n) of 20 years and the fact that improvement is first recouped commercially in year 3 (y).

$$Discounted\ \cos t\ (n,p) = \sum_{m=1}^{n-y+1} \sum_{t=y+m-1}^{n} \left[\frac{1}{1+d}\right]^{t} . AC$$

The discounted contribution (DP) per selected ram in the commercial tier was computed using a similar formula, in which cost was replaced by the contribution rate of selected sires. This rate is a function of the number of progeny per ram and the fact that half of the genes of progeny come from the selected sires. It was assumed that selected rams leave 189 crossbred progeny. The resulting discounted contribution per ram sold was £61.37 per year.

7.3 Results

The overall expected genetic responses in IMF and CFW are shown in Figures 7.1 and 7.2, respectively. Expected genetic gains for CMW are presented in Tables 7.2 and 7.3. The genetic responses of these three traits were calculated, with and without MD_{CT} in the second stage, for the three strategies considered in this study: (1) one-stage selection on ultrasound alone (proportion CT scanned = 0); (2) one-stage selection where all animals are ultrasound and CT scanned (proportion CT scanned = 1); (3) two-stage selection with the proportion of ram lambs CT scanned varying between 0.10 and 0.95. The final selection proportions were 0.05 and 0.60 for ram and ewe lambs, respectively.

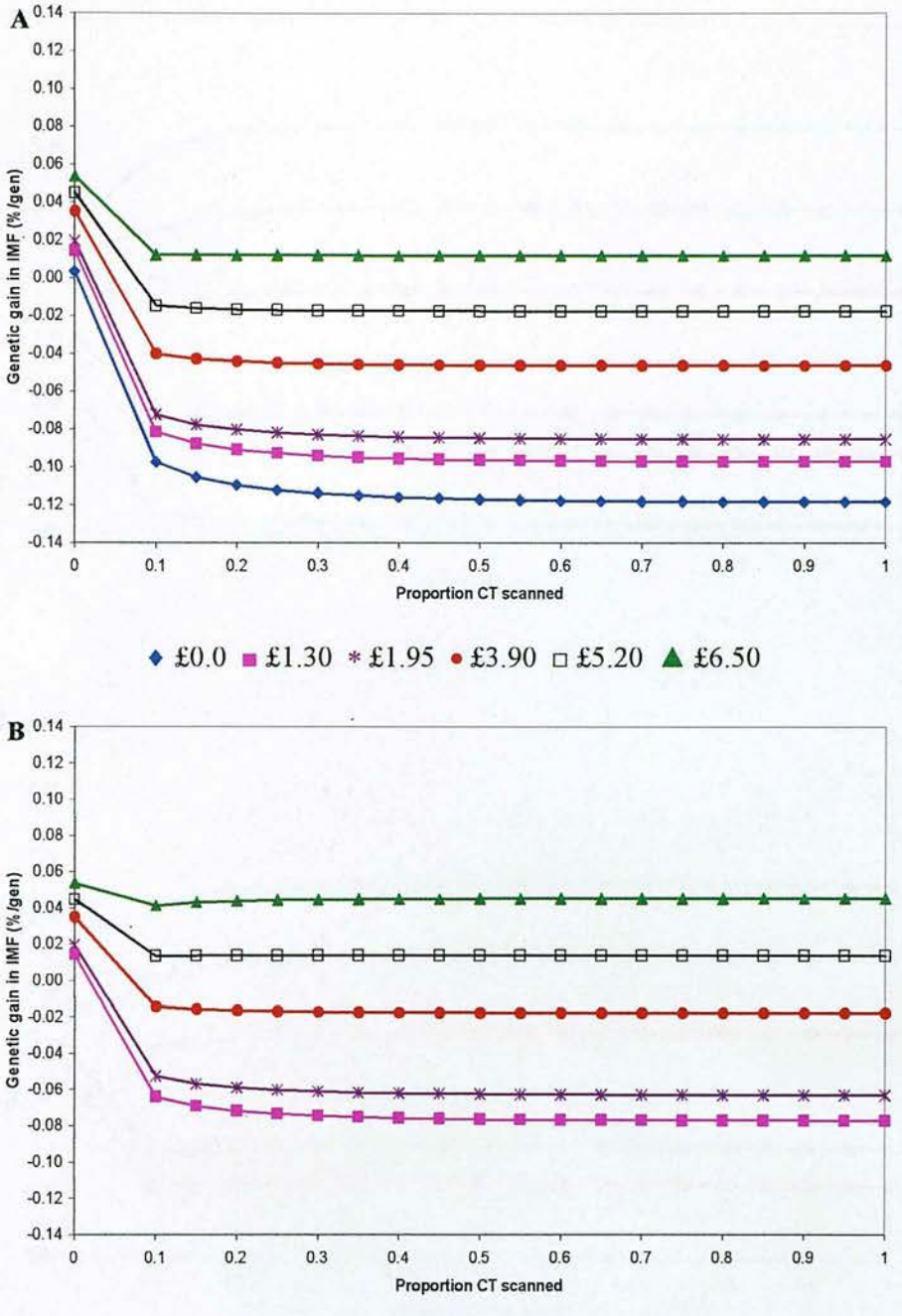


Figure 7.1: Overall genetic responses in IMF per generation from three strategies without (A) and with (B) MD_{CT} as criterion: (1) one-stage selection on ultrasound alone (proportion CT scanned = 0); (2) one-stage selection where all animals are ultrasound and CT scanned (proportion CT scanned = 1); (3) two-stage selection with proportion of ram lambs CT scanned varying between 0.10 and 0.95. The responses correspond to six economic values of IMF, with the economic values of CMW and CFW being £4 and £-1.3, respectively

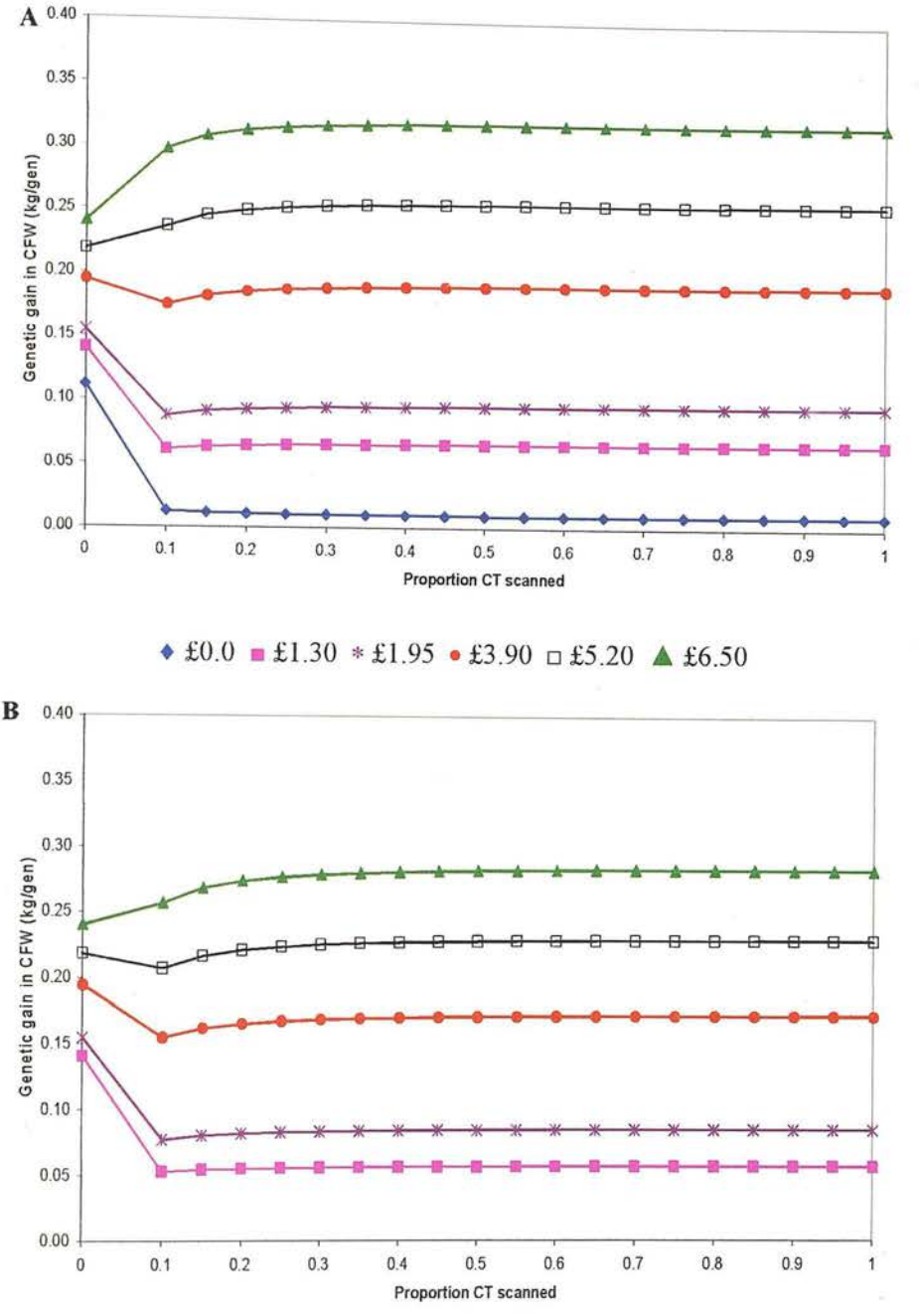


Figure 7.2: Overall genetic responses in CFW per generation from three strategies without (A) and with (B) MD_{CT} as criterion: (1) one-stage selection on ultrasound alone (proportion CT scanned = 0); (2) one-stage selection where all animals are ultrasound and CT scanned (proportion CT scanned = 1); (3) two-stage selection with proportion of ram lambs CT scanned varying between 0.10 and 0.95. The responses correspond to six economic values of IMF, with the economic values of CMW and CFW being £4 and £-1.3, respectively

Table 7.2: Overall genetic responses in CMW per generation from three strategies without MD_{CT} as criterion: (1) one-stage selection on ultrasound alone (proportion CT scanned = 0); (2) one-stage selection where all animals are ultrasound and CT scanned (proportion CT scanned = 1); (3) two-stage selection with proportion of ram lambs CT scanned varying between 0.05 and 0.95. The responses correspond to six economic values of IMF, with the economic values of CMW and CFW being £4 and £-1.3, respectively

Proportion CT scanned	Without MD_{CT} as selection criterion					
	IMF relative economic values					
	0	1.3	1.95	3.9	5.2	6.5
0.00	0.599	0.606	0.609	0.610	0.607	0.601
0.10	0.742	0.770	0.782	0.806	0.808	0.797
0.15	0.791	0.816	0.826	0.843	0.841	0.826
0.20	0.816	0.839	0.848	0.861	0.856	0.839
0.25	0.832	0.853	0.861	0.872	0.865	0.846
0.30	0.842	0.862	0.870	0.878	0.870	0.850
0.35	0.850	0.869	0.875	0.883	0.874	0.853
0.40	0.855	0.873	0.880	0.886	0.876	0.855
0.45	0.859	0.876	0.883	0.888	0.878	0.856
0.50	0.862	0.879	0.885	0.889	0.879	0.857
0.55	0.864	0.880	0.886	0.890	0.879	0.857
0.60	0.866	0.882	0.887	0.891	0.880	0.857
0.65	0.867	0.883	0.888	0.891	0.880	0.857
0.70	0.868	0.883	0.889	0.892	0.880	0.857
0.75	0.869	0.884	0.889	0.892	0.880	0.858
0.80	0.869	0.884	0.889	0.892	0.880	0.858
0.85	0.869	0.884	0.889	0.892	0.880	0.858
0.90	0.869	0.885	0.890	0.892	0.880	0.858
0.95	0.870	0.885	0.890	0.892	0.880	0.858
1.00	0.870	0.885	0.890	0.892	0.880	0.858

Table 7.3: Overall genetic responses in CMW per generation from three strategies with MD_{CT} as criterion: (1) one-stage selection on ultrasound alone (proportion CT scanned = 0); (2) one-stage selection where all animals are ultrasound and CT scanned (proportion CT scanned = 1); (3) two-stage selection with proportion of ram lambs CT scanned varying between 0.05 and 0.95. The responses correspond to six economic values of IMF, with the economic values of CMW and CFW being £4 and £-1.3, respectively

Proportion CT scanned	With MD_{CT} as selection criterion				
	IMF relative economic values				
	1.3	1.95	3.9	5.2	6.5
0.00	0.606	0.609	0.610	0.607	0.601
0.10	0.769	0.778	0.790	0.782	0.762
0.15	0.817	0.824	0.831	0.820	0.797
0.20	0.841	0.847	0.851	0.839	0.815
0.25	0.856	0.861	0.864	0.850	0.824
0.30	0.865	0.871	0.871	0.857	0.830
0.35	0.872	0.877	0.877	0.861	0.834
0.40	0.877	0.882	0.880	0.864	0.837
0.45	0.881	0.885	0.883	0.866	0.839
0.50	0.883	0.888	0.884	0.868	0.841
0.55	0.886	0.889	0.886	0.869	0.842
0.60	0.887	0.891	0.887	0.870	0.842
0.65	0.888	0.892	0.888	0.870	0.843
0.70	0.889	0.892	0.888	0.871	0.843
0.75	0.889	0.893	0.888	0.871	0.843
0.80	0.890	0.893	0.888	0.871	0.843
0.85	0.890	0.893	0.888	0.871	0.843
0.90	0.890	0.894	0.889	0.871	0.843
0.95	0.890	0.894	0.889	0.871	0.843
1.00	0.890	0.894	0.889	0.871	0.843

7.3.1 Genetic responses without including MD_{CT} as selection criterion

Genetic responses in the traits in the breeding goal without including MD_{CT}, and calculated with null economic value for IMF, are those that would be expected in a scenario similar to the current one (Figures 7.1A and 7.2A; Table 7.1). When selection was based only on

ultrasound measurements, the expected genetic gains in CMW and CFW were approximately 0.60 and 0.11 kg/generation, respectively. When CMW_{CT} and CFW_{CT} were included as selection criteria, there was an important increase in the overall gain in CMW compared to that obtained by one stage selection based on ultrasound (25 to 45% depending on the proportion of animals CT scanned), whilst the genetic gain in CFW was closer to zero (values below 0.01 kg/generation). Because of the strong genetic correlation between CFW and IMF, the correlated responses in IMF became negative, with values in the range between -0.01 and -0.12 %/generation.

Increasing economic values for IMF resulted in genetic gains that were closer to zero in IMF in the two-stage strategy, but they remained negative for IMF economic values of £5.20 or below. The genetic responses in CFW also increased almost proportionally to the rise in the economic values of IMF, achieving values higher than the gains in the one-stage strategy with the highest IMF economic values. The genetic responses in CMW increased somewhat when the economic values of IMF were between £1.3 and £3.9, and showed a slight decrease at an IMF economic value of £5.2. Genetic gain for CMW decreased at the maximum economic value considered in this study (£6.50).

7.3.2 Genetic responses when MD_{CT} was included in the second stage of selection

The incorporation of MD_{CT} as a predictor of IMF allowed more favourable genetic responses in IMF (Figure 7.1B) without further unfavourable increases in CFW (Figure 7.2B). For any specific economic value of IMF, genetic responses in CFW were smaller than expected gains obtained when MD_{CT} was not included, whilst the gain in IMF was less negative or became positive.

In terms of CMW, the genetic responses did not change significantly (average difference 0.1%) for economic values of IMF between £1.3 and £3.90 (Table 7.3). Higher economic values tended to reduce slightly (2% on average) the genetic responses in CMW from two-stage selection, but the values remained above those obtained by one-stage selection based on ultrasound.

7.3.3 Economic benefits at different economic values of IMF

The economic responses for the two-stage selection strategies in which MD_{CT} was included are presented in Figure 7.3 for proportions of ram lambs CT scanned ranging from 0.10 to 0.50.

Positive returns were obtained when the proportion of lambs CT scanned was 0.10. The maximum marginal net discounted returns (MNDR) were reached when the proportions of rams CT scanned were 0.15, for all combinations of economic values, and then declined as numbers CT scanned increased further.

The highest MNDR corresponded to the selection index that did not include IMF (economic value of zero), with a maximum values of £590,000. Higher economic values for IMF were associated with lower economic returns. The lowest maximum return was £340,000 for an IMF economic value of £6.50.

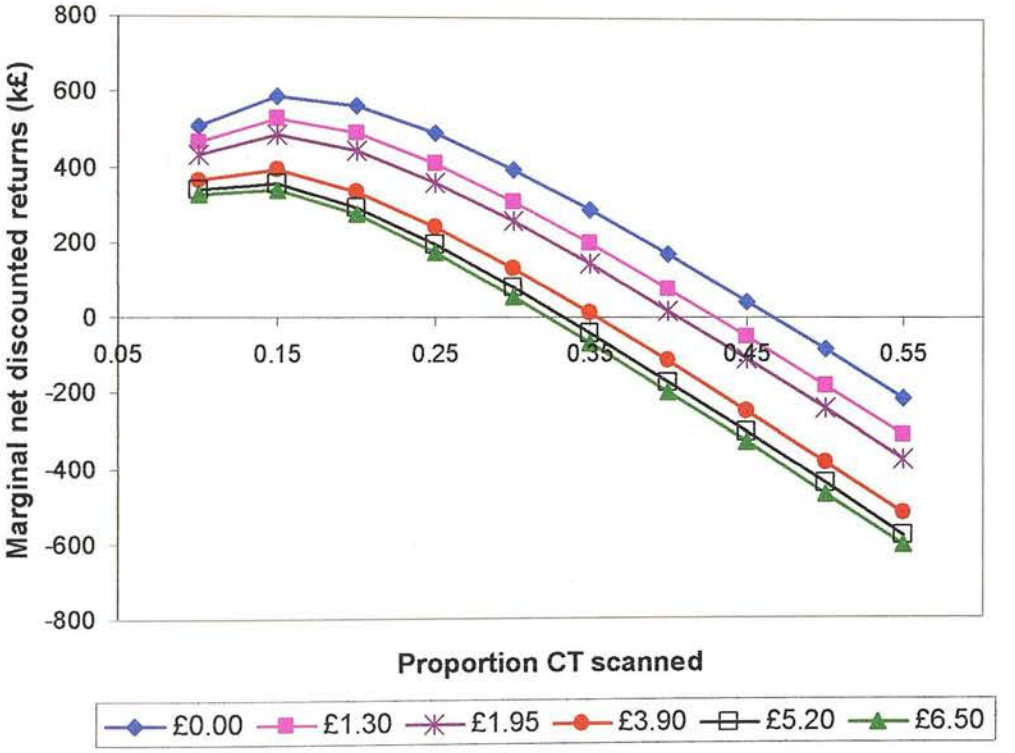


Figure 7.3: Expected marginal net discounted returns over 20 years for different economic values of IMF, whilst the values of CMW and CFW were £4/kg and £-1.3/kg, and assuming that the top 5% rams were selected

7.4 Discussion

Concerns regarding the antagonism between selecting for leaner carcasses and maintaining or improving meat eating quality have been raised in lamb (Gardner *et al.*, 2006;

Karamichou *et al.*, 2006b) and pork (Schwab *et al.*, 2006). The possibility of preventing or limiting the reduction in IMF, whilst decreasing total fat, was investigated by including an *in vivo* measurement of IMF using CT.

7.4.1 Expected genetic responses for lean growth index

In order to evaluate the effect of selection for the lean growth index on IMF, the expected response in this trait, and in CMW and CFW, were calculated in both one-stage and two-stage selection programmes with null economic value for IMF. The expected gains for CMW and CFW are similar to those reported by Young *et al.* (2001a). When selection was based exclusively on ultrasound measurements, IMF increased as a consequence of the positive genetic gain in CFW. The inclusion of CT measurements of fat and muscle weights as criteria led to more favourable expected genetic gains in CFW and CMW, which is in agreement with previous studies by Young *et al.* (2001a) for terminal breeds in the UK, and Jopson *et al.* (1995) for sheep breeding programmes in New Zealand. Negative genetic responses in IMF were expected as CFW decreased due to the positive correlation between these two traits.

When IMF was included in the breeding objective, increasing economic values for IMF were associated with increasing genetic gains in IMF, and consequently in CFW. In a one-stage selection programme, this represents larger positive genetic gains in IMF, but also much fatter carcasses because of the larger genetic gains in CFW. In a two-selection strategy, increasing the economic values of IMF had the favourable effect of diminishing the genetic reductions in IMF, which became closer to zero. However, increasing the emphasis on IMF in the breeding objective had unfavourable consequences on carcass fat content. Genetic responses in CFW tended to be similar or higher to those predicted in a one-stage selection strategy when IMF economic values increased.

7.4.2 Inclusion of MD_{CT} in the selection index in a two-selection programme

The value of MD_{CT} as a predictor of IMF has been reported only recently, and there are few studies on this new *in vivo* tool (Young *et al.*, 2001a; Karamichou *et al.*, 2006b). The phenotypic association between MD_{CT} and IMF used in this study is moderate. Macfarlane *et al.* (2005) found higher values of accuracy ($R^2 = 0.57$) for the prediction of IMF using CT information, but this prediction included other fat traits, such as fat areas measured in the CT images, in addition to MD_{CT}. Because of the strong association between the different fat depots, inclusion of more than one may improve the accuracy of the prediction of IMF but it

may result in stronger correlated responses in carcass fat.

The utilisation of MD_{CT} as a selection criterion combined with CFW_{CT} and CMW_{CT} showed that it is possible to minimise the reduction of IMF, with a minimal increase in the genetic gain in CFW, whilst CMW was not significantly affected.

Only measurements taken on the selection candidates were considered in this study, which leads to more conservative estimations of genetic responses and economic gains. The inclusion of measurements on relatives increases the accuracy of selection and therefore improves the rates of genetic gains in general. However, similar trends in genetic responses in the different traits, with and without phenotypic information from relatives, are expected because the heritabilities of selection criteria and breeding goal traits are all moderate to high (Simm and Dingwall, 1989).

Because of the negative genetic associations between IMF and carcass quality, measured in terms of CFW and CMW, the improvement in one characteristic will reduce the genetic progress in the other.

The emphasis to be given to IMF thorough its relative economic value can be evaluated based on the desired responses in all traits included in the breeding objective. Different scenarios can be considered. Taking a two-stage selection strategy with the best 15% of selection candidates being CT scanned, an economic value of £3.9 for IMF will determine a minimum reduction in IMF of –0.02 %/generation and an intermediate increase of CFW of approximately 0.16 kg/generation, whilst CMW will improve at the maximum gain that can be achieved with this proportion of animals selected (0.83 kg/generation). At the same proportion of ram lambs selected for CT scanning and with economic values between £5.20 and £6.50, IMF shows positive gains but this will coincide with higher gains in CFW, ranging between 0.22 and 0.27 kg/generation. The correlated response in CMW will decrease to values between 0.82 and 0.80 kg/generation.

The proportion of ram lambs being selected to go forward for CT scanning has a major influence on the economic returns from a two-stage selection strategy. In the present study, the maximum economic benefits were obtained for all IMF economic values when the proportions of lambs CT scanned were 0.15. This is in agreement with results reported by studies of two-stage breeding programmes for carcass muscle and fat weight alone (Jopson *et*

al., 1997; Young *et al.*, 2001a) in which similar selection intensities led to the maximum returns.

The cost of CT scanning assumed in this study was £65, which is equivalent to the commercial cost per animal to get the information on CMW_{CT} and CFW_{CT}. Obtaining MD_{CT} from the same CT images used to predict CMW_{CT} and CFW_{CT} does not increase the cost because there is no need for further image collection or analysis, which also facilitates processing this additional information from a logistical point of view.

The economic values of IMF influenced not only the genetic gains of traits in the breeding objectives but also the economic returns from a two-stage selection strategy. Although maximum returns were achieved at similar proportions of animals CT scanned, independently of the economic values of IMF, MNDR decreased when the economic values of IMF increased. This may be due to the unfavourable correlation between IMF and CFW.

Because there are no quantitative market signals regarding the economic value of eating quality or IMF in lambs, arbitrary values for IMF were considered in this study. It was also assumed that the economic relevance of IMF is via a linear association with eating quality. However, the economic importance of IMF, explained by its association with eating quality, may not be linear. In this case, eating quality will be adversely affected when a threshold is reached as the result of the selection for leaner carcasses. The implementation of strategies to avoid this seems prudent, including also the use of restricted selection indices. Nevertheless, more information on the associations among CTMD, IMF and the eating quality traits relevant for consumers is needed.

The results obtained in this study indicate that inclusion of MD_{CT} may be an effective tool to minimise the reduction of IMF and possible unfavourable effects on eating quality, and to produce leaner carcasses at the same time. More favourable genetic progress could be achieved by developing ways to assess IMF on farms, which would allow the selection for this trait also in the first stage. The use of ultrasound is an option worth exploring further. The use of molecular information is another option to "break" the unfavourable correlation between CFW and IMF. For example, a quantitative trait locus (QTL) affecting IMF but with no effect on other fat depots has been found in pigs (Duthie *et al.*, 2007).

7.5 Conclusions

In summary, the results of this study indicate that selection based on the lean growth index assumed may decrease the content of IMF as a correlated response, mainly due to the strong correlation between total fat and IMF. The negative genetic response in IMF, which may affect the eating quality of lamb, can be reduced by including MD_{CT} as a selection criterion. From a practical point of view, MD_{CT} is a measurement that can be easily obtained from animals already being CT scanned. However, more research is required on heritabilities of, and genetic and phenotypic associations among, IMF, MD_{CT} and eating quality in lambs. Future molecular information may contribute to reduce carcass fatness without compromising the content of IMF and eating quality.

Chapter 8: General Discussion

8.1 Introduction

Genetic improvement of carcass and meat quality traits can make an important contribution to the production of lean lamb carcasses with high conformation scores, meeting market specifications, while meeting the eating quality expected by consumers.

In this thesis, the development of tools to genetically improve muscularity and eating quality, based on *in vivo* measurements using computed tomography (CT), was explored in two important and divergent sheep breeds (Texel, TEX; Scottish Blackface, SBF). The first aim was the development of a comprehensive method to assess muscularity *in vivo* in lambs, which has been presented and validated in these two breeds. This approach was based on *in vivo* muscularity indices that were calculated based on new image analysis procedures for spiral CT scans (SCTS) (Chapter 3 and Chapter 4). Secondly, the associations among the *in vivo* muscularity indices and with other carcass quality traits and eating quality were investigated (Chapter 4 and Chapter 5), and genetic parameters were estimated for these and other CT traits relevant to current sheep breeding programmes (Chapter 6). Additionally, the relationships between CT muscle density, a novel CT predictor of intramuscular fat (IMF)/ meat eating quality, muscularity and carcass composition assessed by CT were investigated (Chapter 6). Finally, the consequences of breeding for leaner carcasses on the IMF content, and therefore meat eating quality, were quantified in a simulation study (Chapter 7). This also evaluated the effects of incorporating CT muscle density on the genetic improvement of lamb carcass composition and IMF.

This final chapter summarises the main findings of this thesis, described in the previous chapters, and discusses them in the context of the lamb meat industry. Possible additional developments of the *in vivo* measurements using CT and areas for future research are also discussed here.

8.2 *In vivo* muscularity indices

As conventional carcass conformation classification is confounded with carcass fatness (especially in breeds with higher fat content), improving conformation without increasing fat content requires measures of the shape of the carcass and muscle regions, which are independent of the degree of fatness.

Objective *in vivo* measures of muscularity, based on an approach that was previously only feasible on carcasses (Purchas *et al.*, 1991), were developed using SCTS. These included

linear dimensions (skeletal lengths) and muscle volumes (in selected regions or the whole carcass). As well as considering the carcass as a whole, the work focused on the hind leg and lumbar region, which are the most economically important regions of lamb carcasses.

An automatic image analysis method has been developed for SCTS, to calculate the volume of muscle in the hind leg and lumbar region in lambs. This involved the development of an automated method of segmenting the muscle tissue from non-muscle tissue in all contiguous cross-sectional scans within these regions (Chapter 3). Muscle areas were then measured and multiplied by the inter-scan distance to calculate muscle volumes. These volume measurements predicted dissected muscle weights very accurately (R^2~0.85 for lumbar region; R^2~0.97 for hind leg).

The computation of muscularity indices also requires the measurement of skeletal dimensions. Therefore, a method was developed to measure the lengths of the femur bone using SCTS (Chapter 4), whilst the measurement of the spine length was done on the topograms as previously described by Jones *et al.* (2002). These measurements, combined with the muscle volumes, produced new muscularity indices for the hind leg ($HLMI_{CT}$) and lumbar region ($LRMI_{CT}$), as well as that for the whole carcass (CMI_{CT}), which objectively describe muscularity.

Results of Chapter 4 indicate that these indices agreed well with similar indices produced using dissected muscle weights and skeletal dimensions, with the highest accuracy in the hind leg region. These indices will be useful for the *in vivo* identification of selection candidates with superior muscularity in the higher-priced regions of the carcass.

8.2.1 Muscle mass and skeletal dimensions using SCTS

Accuracy for the $HLMI_{CT}$ was higher compared to muscularity indices for the loin region as the accuracies of both of its components, muscle volume and femur length, using SCTS were very high. An important contribution to this, compared to previous studies (before SCTS was available at SAC), was the utilisation of the potential of the SCTS to measure femur length as described in Chapter 4. Measuring the length of the femur as described was originally only possible using the computer and the software associated with the CT scanner, which restricted the implementation of the measurements from a practical point of view, as measurements could only be made when the CT scanner was not in use. Therefore, new PC software has been developed to obtain the same display of SCTS in three dimensions (3D)

that enables accurate measurement of femur length 'offline'.

The lower accuracy of the $LRMI_{CT}$ compared to $HLMI_{CT}$ was probably due to: (i) the fact that all muscles were considered in the dissection index, whilst only the *m. longissimus* and *m. multifidus* were included in the CT index and (ii) the lower accuracy of the CT spine length measures. Problems identifing the vertebrae, especially in smaller lambs, could explain the lower accuracy of measuring the spine length (Jones *et al.*, 2002). Improving this measure may also improve the accuracy of this index. The SCTS may also provide an alternative option to assess spine length by identifying the cross-sectional images with the landmarks for the spine, counting the number of slices, and then multiplying this number by the distance between slices. Although this alternative needs to be investigated furher, it may provide a more repeatable measurement, which may improve accuracy.

The automated image analysis procedure developed for SCTS (Chapter 3) was shown to be an accurate tool to measure *in vivo* muscle volume in the hind leg and lumbar region of lambs. This allows rapid analysis of the large number of cross-sectional images contained in SCTS (approximately 110 images for a SCTS of a 35 kg lamb covering the hind leg, lumbar region and shoulder) with high repeatability, which is relevant for the practical application of this tool in breeding programmes.

As shown in Chapter 3, this method provides an accurate measurement of muscle mass for two regions of the carcass. Further developments in the automation of the image analysis program may allow measurements of the three main tissues (fat, muscle and bone) in all regions of the body. In addition, refinements of CT image analysis of the hip region may allow specific measurements of muscle mass and muscularity of the leg and chump, which are the main joints within the hind leg. Removal of the non-carcass components from the SCTS images can be performed independently on each cross-sectional image, except in the hip region, where information from multiple scans, i.e. 3D information, might improve the quality of the image analysis. The algorithm has been implemented in STAR more recently and used to segment images from the hip region, producing results comparable with those from manual interpretation by a skilled operator. However, information on dissected muscle weights specific for the leg and chump were not available to re-evaluate the accuracy of the CT image analysis.

The accurate calculation of muscle volume in the different carcass regions could also

become a useful tool for assessing meat yield in the most valuable joints, and potentially for selection for higher meat yield in the regions of interest. This could be relevant in the near future if measuring meat yield in the different regions becomes more common in abattoirs with the implementation of VIA/VISA (video image [scanning and] analysis) systems.

The use of SCTS potentially allows this information to be obtained *in vivo* without the need for developing prediction equations. Although the accuracy of predictions of carcass composition is moderate to high for the different tissues (Young *et al.*, 2001; Macfarlane, 2006), prediction equations may require regular updating because of changes of quantity, and possibly distribution, of the different tissues in the body, as result of genetic improvement of carcass composition.

8.3 Selection for improved muscularity

Improving muscularity has been considered beneficial for the sheep industry because of: (i) its effect on the shape of the cut, which influences its appeal to consumers, and (ii) its favourable association with carcass conformation and fatness scores, becoming a valuable selection trait for the improvement of conformation without increasing fatness.

The *in vivo* muscularity indices were positively correlated, in general, with carcass conformation score (the highest correlations were with $HLMI_{CT}$ in both breeds), dissected muscle weight and muscle to bone ratio, at the phenotypic level (Chapter 4). When adjustments were made for carcass weight, muscularity was not positively correlated in either breed with dissected carcass fat weight or carcass fatness score. Results described in Chapter 4 suggest that CT muscularity indices are an accurate method to improve carcass conformation, providing measurements that, at a constant carcass weight, are independent of carcass fatness.

Muscularity indices in different body regions were moderately to highly heritable in both TEX and SBF breeds and positively genetically correlated with each other, although the strength of these correlations differed between breeds (Chapter 4). The need for multiple muscularity indices may vary between breeds, and it may also depend on the relative economic importance of improving muscularity of the different regions. Although the economic value of muscularity has not been quantified, the strong association of $HLMI_{CT}$ with conformation scores and its higher accuracy, in conjunction with the positive correlation among muscularity of the different regions, suggests it would be useful to include

this muscularity index in a multi-trait index.

In order to evaluate the consequences of selection for muscularity in addition to the other carcass traits relevant for the different breeds, accurate estimations of genetic parameters are needed, particularly genetic correlations. Information on economic values of the different traits is also poor in general, complicating the derivation of the correct weights for the different traits. in a multi-trait index.

Differences in muscularity between breeds (TEX and SBF) and between progeny of high- and low-muscularity sires (HM, LM) within breed, were quantified using the new muscularity indices (Chapter 5). Significant breed differences were found, and also between lambs sired by HM and LM rams (differences were around 4% for both regions).

Results in Chapter 5 indicate that selecting for improved muscularity in lambs is expected to be neutral with respect to meat eating quality. No phenotypic effect of muscularity on meat eating quality has been found in TEX or SBF lambs. The linear associations between muscularity of the hind leg and lumbar region, within each breed, and eating quality evaluated by taste panel on meat samples from both regions were not statistically significant. Although breed differences were identified in eating quality (meat of SBF lambs was more tender, with a stronger lamb flavour, and a higher overall liking), within breed eating quality traits were not significantly affected by sire group. Although this lack of differences between the progeny of HM and LM sires suggests that there are no direct genetic effects of muscularity on eating quality, it would be important to confirm this by the estimation of genetic parameters among these traits.

8.4 Use of CT muscle density in sheep breeding programmes

The *in vivo* CT measurement of muscle density is a promising technique for the selection of live animals for IMF content, and hence improved meat eating quality. Within a certain range, IMF is known to have a positive effect on meat eating quality of red meat (e.g. Savell and Cross, 1988), although less information is available on this relationship in lamb meat, compared to meat from other livestock species (i.e. beef).

CT muscle density measured *in vivo*, which was moderately to highly heritable, had strong negative genetic and phenotypic associations with IMF in both TEX and SBF breeds, as well as with total carcass fat weight predicted by CT (Chapter 6). This confirms the potential of

CT muscle density as a selection trait for the genetic manipulation of IMF, and indirectly of eating quality. The estimated parameters also indicate an antagonism between the aims of reducing carcass fatness but not decreasing IMF and/or eating quality.

Little association was found between muscularity indices and CT muscle weight and CT muscle density (Chapter 6), implying that selection for CT muscle density would not have a negative effect on muscularity or muscle weight.

Although CT muscle density could be used to improve meat quality traits through its association with IMF, the complexity of eating quality (i.e. the high number of attributes which may interact), the lack of genetic parameters for these traits and difficulties of quantifying their economic relevance, make it extremely difficult to evaluate CT muscle density as a tool to improve eating quality. However, in the current context and with the information available, the results of Chapter 7 indicate the incorporation of CT muscle density as a selection criterion in a two-stage selection programme would minimise the decrease of IMF due to the reduction of carcass fat when improving carcass quality.

This would help to ensure that the levels of IMF remain above the critical levels for consumers' satisfaction in order to achieve a palatable and healthy product. Values of 2 to 3% of IMF were suggested by Savell and Cross (1988), although it will be important to confirm this threshold, as well as the associations among IMF, different eating quality attributes and consumers' satisfaction, specifically for lamb meat and in target markets.

The lack of clear signals from the market regarding the economic relevance of different carcass and meat quality traits not only reduces the motivation of breeders and farmers to make use of the technical tools available to improve quality, but also may limits the possible approaches for the optimisation of breeding programmes, specially when there are several relevant traits in the breeding objectives.

The expected gains from including CT muscle density depend on the desired gains by the industry in all traits in the breeding objective. However, positive returns for the industry from using CT muscle density at the second stage of selection were predicted for all economic values of IMF (Chapter 7). Maximum benefits for the industry were obtained when the proportions of ram lambs CT scanned are 0.15.

8.5 Future research

8.5.1 Larger data sets

The genetic parameters (heritabilities and genetic correlations) estimated in this study tend to have large associated standard errors, due to the relatively small data set and the shallow pedigree information available, especially for the SBF lambs. The project that provided the experimental data was originally designed to look at general relationships between muscularity and meat quality traits and not to provide precise estimates of genetic parameters, although the genetic results produced add further understanding of these associations. Although these estimates allow some insight into the genetic control of the traits of interest and the direction and general magnitude of their relationships with other important traits, improving the accuracy of these estimates is important.

The development of automated image analysis methods for SCTS was stimulated by the need to obtain measurements on a large number of animals. Data management procedures for the large volume of information obtained from the segmentation were also implemented. The development of methods for measuring the length of the femur independently of the CT scanner associated computer and software, using new PC-software also had this aim. These new tools will facilitate recording these traits for potential future utilisation in breeding programmes as well as in other research projects. The automated procedures and advantages of SCTS may also allow a more comprehensive understanding of the relationships among muscle content, skeletal dimensions and conformation, and their implication from a genetic point of view.

Now that these accurate, largely automated methods for *in vivo* estimation of muscularity using CT have been developed and evaluated, these traits should be measured in larger numbers of lambs, of different breeds, to allow precise estimation of relevant genetic parameters for these traits. This would provide the accurate information needed for the incorporation of these muscularity traits into breeding programmes in different breeds.

8.5.2 Association with other economically relevant traits

Genetic relationships with all other relevant traits for each breed, not measured in the present study, will need to be investigated to check for unforeseen side effects of selection on these traits. For example, maternal traits feature heavily in the breeding objectives for hill or upland breeds and associations between these traits and predictors of muscularity or meat quality traits must also be known before these traits can be combined in any selection index

for such breeds. The improvement of muscularity may be associated with a higher incidence of birth difficulties. This should be evaluated in terms of not only consequences on production and animal welfare but also the economic impact, which may differ among breeds and environments.

As VISA systems are being evaluated in the UK abattoirs as the future basis of a value-based marketing system, relationships between CT muscularity and other CT traits and VISA characteristics should be investigated. If it is possible to measure also fat and muscle weights with SCTS, using similar image analysis to the one applied in this study for muscle, CT could also provide useful information for the calibration of VISA systems, which is being investigated in SAC.

8.5.3 CT muscle density, IMF and eating quality

In addition to the improvement of the accuracy of genetic parameters for CT muscle density, IMF and eating quality, it is important to highlight the relevance of further investigations of the associations among them. For example, the relationship of IMF and the different aspects of eating quality should be confirmed in lambs of the main breeds in the UK, and under commercial conditions and post- mortem treatments used in the UK. A better understanding of the association between IMF and eating quality will provide also relevant information to improve selection indices.

CT muscle density may also be able to predict histology traits because the muscle fibre characteristics may affect the real muscle density. Hence, the effect of muscularity on histology traits, as well as the associations among histology traits, eating quality and CT muscle density, should be explored.

8.6 Conclusions

An accurate, reliable method for measuring muscularity in live lambs by CT was developed in this study. The proposed CT muscularity indices for the hind leg and lumbar region have high and moderate accuracies, respectively, in the two contrasting breeds (TEX and SBF). Selection for improved muscularity of sheep based on the proposed CT indices is likely to be positive for carcass quality and neutral with respect to meat eating quality.

Muscle density measured by CT is a promising technique for the selection of live animals for IMF content, and hence improved meat eating quality. This *in vivo* measurement is also a

useful tool to minimise any possible negative effect of selection for leanness on eating quality, due to undesirable reductions on IMF.

Results from the new *in vivo* measurements presented here suggest that including new carcass and meat quality traits in sheep breeding programmes may complement the current traits under selection. The information available indicates that CT can be used to genetically improve these new traits, although estimates of genetic parameters need to be confirmed in larger data sets. The implementation and utilisation of these tools would be stimulated by the existence of value-based marketing systems that reward carcass and meat eating quality.

References

Abdullah, A.Y., Purchas, R.W., Davies, A.S. and Kirton, A. H. 1993. Relationship between objective and subjective measurements of carcass muscularity. *Proceedings of the New Zealand Society of Animal Production* **53**: 397–402.

Arsenos, G., Banos, G., Fortomaris, P, Katsaounis, N., Stamataris, C., Tsaras, L., and Zygoyiannis, D. 2002. Eating quality of lamb meat: effect of breed, sex, degree of maturity and nutritional management. *Meat Science* **60**: 379-387.

Brenoe, U.T. and Kolstad, K. 2000. Body composition and development measured repeatedly by computer tomography during growth in two types of turkeys. *Poultry Science* **79**: 546-552.

Burrow, H.M., Moore, S.S., Johnston, D.J., Barendese, W. and Bindon B.M. 2001. Quantitative and molecular genetic influence on properties of beef: a review. *Australian Journal of Experimental Agriculture* **41**: 893-919.

Butler-Hogg, B.W., Francombe, M.A. and Dransfield, E. 1984. Carcass and meat quality of ram and ewe lambs. *Animal Production* **39**: 107-113.

Campbell, A.W. and Waldron, D.F. 2006. Genetic improvement of meat production in small ruminants. *Proceedings of the 8th World Congress of Genetics Applied to Livestock Production*, CD-ROM communication n° 04-06.

Carson, A.F., Irwin, D. and Kilpatrick, D.J. 2001a. A comparison of Scottish Blackface and Cheviots ewes and five sire breeds in terms of lamb output at weaning in hill sheep systems. *Journal of Agricultural Science* **137**: 221-233.

Carson, A.F., Moss, B.W., Dawson, L.E.R. and Kilpatrick D.J. 2001b. Effects of genotype and dietary forage to concentrate ratio during the finishing period on carcass characteristics and meat quality of lambs from hill sheep systems. *Journal of Agricultural Science* **137**: 205-220.

CEC (Commission of the European Communities) 2002. *Report from the Commission to the Council on the implementation of Council Regulation (EEC) No. 2137/92 concerning the Community scale for the classification of carcasses of ovine animals.* <http://europa.eu.int/eur-lex/lex/LexUriServ/site/en/com/2002/com2002_0295en01.pdf>.

Chambers, E. and Bowers, J.R. 1993. Consumer perceptions of sensory qualities in muscle foods. *Food technology* **47**: 116-120.

Cohen, J. 1969. *Statistical power analysis for behavioural sciences*. New York: Academic Press.

Conington, J., Bishop, S.C., Grundy, B., Waterhouse, A. and Simm, G. 2001. Multi-trait selection indexes for UK sustainable hill sheep. *Animal Science* **73**: 413-424.

Conington, J. Bishop, S.C., Lambe, N.R., Bünger, L. and Simm, G. 2006a. Testing selection indices for sustainable hill sheep production – lamb growth and carcass traits. Animal Science 82: 445-453.

Conington, J., Lambe, N., Bünger, L., McLean, K., Bishop, S. and Simm, G. 2006b. Evaluation of responses to multi-trait selection indexes and genetic parameters for computer

tomography-derived carcass traits in UK hill sheep. *Proceedings of the 8th World Congress of Genetics Applied to Livestock Production*, CD-ROM communication n° 04-19.

De Boer, H., Dumont, B.L., Pomeroy, R.W. and Weniger, J.H. 1974. Manual on EAAP reference methods for the assessment of carcass characteristics in cattle. *Livestock Production Science* **1**: 151-164.

de Vries, A.G., Faucitano, L., Sosnicki, A. and Plastow, G.S. 2000. Influence of genetics on pork quality. In *Quality of Meat and Fat in Pigs as affected by Genetics and Nutrition* (eds Wenk C., Fernandez, J.A. and Dupuis M.), pp. 27 – 35. Proceedings of the joint session of the EAAP commissions on pig production, animal genetics and animal nutrition, Zurich, August 1999.

Defra, 2005. *Agricultural Quick Statistics*. http://statistics. defra.gov. uk/esg/quick/agri. asp; Table 8.1.

Dewar-Durie, A. 2000. *The Scottish sheep industry: A way forward*. Report to the Minister of Rural Affairs, Ross Finnie. <http://www.scotland.gov.uk/library3/agri/tssi-01.asp>.

Dikeman, M.E. 1987. Fat reduction in animals and the effects on palatability and consumer acceptance of meat products. *Proceedings of the Reciprocal Meat Conference* **40**: 93-103.

Doran, O., Moule, S.K., Teye, G.A., Whittington, F.M., Hallett, K.G. and Wood, J.D. 2006. A reduced protein diet induces stearoyl-CoA desaturase protein expression in pig muscle but not in subcutaneous adipose tissue: relationship with intramuscular lipid formation. *British Journal of Nutrition* **95**: 609-617.

Dransfield, E., Nute, G.R., Hogg, B.W. and Walters, B.R. 1990. Carcass and eating quality of ram, castrated ram and ewe lambs. *Animal Production* **50**: 291-299.

Duthie, C., Simm, G., Knap, P., Wilson, A., Kalm, E. and Roehe, R. 2007. Novel quantitative trait loci for chemical body composition traits in pigs. *Proceedings of the British Society of Animal Science* **2007**: 3.

Ellis, M., Webster, G.M., Merrell, B.G. and Brown, I. 1997. The influence of terminal sire breed on carcass composition and eating quality of crossbred lambs. *Animal Science* **64**: 77–86.

Falconer, D.S. and Mackay, T.F.C. 1996. *Introduction to quantitative genetics*. 4th edition. Prentice Hall, Harlow, England.

Fisher, A.V. and De Boer, H. 1994. The EAAP standard method of sheep carcass assessment. Carcass measurements and dissection procedures Report of the EAAP Working Group on Carcass Evaluation, in cooperation with the CIHEAM Instituto Agronomico Mediterraneo of Zaragoza and the CEC Directorate General for Agriculture in Brussels. *Livestock Production Science* **38**: 149–159.

Freking, B.A., Keele, J.W., Shackelford, S.D., Wheeler, T.L., Koohmaraie, M., Nielsen, M.K. and Leymaster, K.A. 1999. Evaluation of the Ovine Callipyge Locus: III Genotypic effects on Meat quality traits. *Journal of Animal Science* **77**: 2336-2344.

Fullerton, G.D. 1980. Tissue Imaging and Characterisation. In *Medical Physics of CT and*

Ultrasound (eds Fullerton, G.D. and Zagzebski, J.A.), pp. 125-162. Medical Physics Monograph, American Institute of Physics.

Gardner, G.E., Pethick, D.W. Hopkins, D.L, Hegarty, R.S., Cake, M.A., Boyce, M.D. and Allingham, P.G. 2006. The impact of carcase estimated breeding values on yield and quality of sheep meat. *Proceedings of the 2006 Australian Sheep Industry CRC Conference*: 50-56.

Genstat Committee, 2004. *Genstat 7 release 7.2 (PC/Windows NT)*. Lawes Agricultural Trust, Rothamsted Experimental Station, Harpenden.

Gilmour, A.R., Gogel, B.J., Cullis, B.R., Welham, S.J. and Thompson, R. 2002. *ASREML User Guide Release 1.0*. VSN International Ltd., Hemel Hempstead, UK.

Glasbey, C.A. and Horgan, G.W. 1995. *Image analysis for the biological sciences*. Wiley, Chichester.

Glasbey, C.A., Navajas, E., McLean, K.A., Fisher, A.V., Lambe, N.R., Bünger, L. and Simm, G. 2004. Estimation of muscle volume by automated image analysis of spiral computed tomography scans in sheep. *Proceedings of the British Society of Animal Science* **2004**: 37.

Glasbey, C.A. and Robinson, C.D. 2002. Estimators of tissue proportions from X-ray CT images. *Biometrics* **58**: 928-936.

Glasbey, C.A. and Young, M.J. 2002. Maximum a *posteriori* estimation of image boundaries by dynamic programming. *Applied Statistics* **51**: 209-221.

Goddard, M.E. and Hayes, B. 2002. Optimisation of response using molecular data. *Proceedings of the 7th World Congress of Genetics Applied to Livestock Production*, CD-ROM communication n° 22-01.

Goodpaster, B.H., Kelley, D.E., Thaete, F.L., He, J. and Ross, R. 2000. Skeletal muscle attenuation determined by computed tomography is associated with skeletal muscle lipid content. *Journal of Applied Physiology* **89**: 104-110.

Hassen, A., Wilson, D.E. and Rouse, G.H. 2003. Estimation of genetic parameters for ultrasound-predicted percentage of intramuscular fat in Angus cattle using random regression models. *Journal of Animal Science* **81**: 35-45.

Higgs, J.D. 2000. The changing nature of red meat: 20 years of improving nutritional quality. *Trends in Food Science and Technology* **11**: 85 -95.

Hocquette, J.F. and Gigli, S. 2005. The challenge of quality. In *Indicators of milk and beef quality* (eds Hocquette, J.F. and Gigli, S.), EAAP publication 112, pp. 13-22. Wageningen Academic Publishers, Wageningen, The Netherlands.

Holloway, I.J., Purchas, R.W., Power, M.T. and Thomson, N.A. 1994. A comparison of the carcass and meat quality of Awassi-cross and Texel-cross ram lambs. *Proceedings of the New Zealand Society of Animal Production* **54**: 209-213.

Hopkins, D.L. 1996. The relationship between muscularity, muscle:bone ratio and cut

dimensions in male and female lamb carcasses and the measurement of muscularity using image analysis. *Meat Science* **44**: 307–317.

Hopkins, D.L., Fogarty, N.M. and Menzies, D.J. 1997. Differences in composition, muscularity, muscle:bone ratio and cut dimensions between six lamb genotypes. *Meat Science* **45**: 439-450.

Hopkins, D.L., Hegarty, R.S. and Farrell, T.C. 2005. Relationships between sire estimated breeding values and the meat and eating quality of meat from their progeny grown on two planes of nutrition. *Australian Journal of Experimental Agriculture* **45**: 525-533.

Imaginis. 2005. *Computed tomography imaging (CT scan, CAT scan): spiral CT and helical CT*. http://imaginis.com//ct-scan/spiral.asp

Jackson, S.P., Miller, M.F. and Green, R.D. 1997. Phenotypic characterization of Rambouillet sheep expressing the Callipyge gene. II. Carcass characteristics and retail yield. *Journal of Animal Science* **75**: 125-132.

Jackson, S. and Thomas, R. 2004. *Cross-Sectional Imaging*. Churchill, Livingstone.

Johnson, P.L. 2003. *A direct search for QTL affecting carcass composition traits in Texel sheep*. PhD Thesis. Massey University.

Johnson, P.L., McEwan, J.C., Dodds, K.G., Purchas, R.W. and Blair, H.T. 2005a. Meat quality traits were unaffected by a quantitative trait locus affecting leg composition traits in Texel sheep. *Journal of Animal Science* **83**: 2729 –2735.

Johnson, P.L., Purchas, R.W., McEwan, J.C. and Blair, H.T. 2005b. Carcass composition and meat quality differences between pasture-reared ewe and ram lambs. *Meat Science* **71**: 383–391.

Jones, H.E., Lewis, R.M. and Warkup, C.C. 2003. Market requirements for lamb. *British Food Journal* **105**: 364-379.

Jones, H.E., Lewis, R.M., Young, M.J. and Simm, G. 2004. Genetic parameters for carcass composition and muscularity in sheep measured by X-ray computer tomography, ultrasound and dissection. *Livestock Production Science* **90**: 167-179.

Jones, H.E., Lewis, R.M., Young, M.J. and Wolf, B.T. 2002. The use of X-ray computer tomography for measuring the muscularity of live sheep. *Animal Science* **75**: 387-399.

Jones, H.E., Simm, G., Dingwall, W.S. and Lewis, R.M. 1999. Genetic relationships between visual and objective measures of carcass composition in crossbred lambs. *Animal Science* **69**: 553-561.

Jopson, N.B., Amer, P.R. and McEwan, J.C. 2004. Comparison of two-stage selection breeding programmes for terminal sire sheep. *Proceedings of the New Zealand Society of Animal Production* **64**: 212-216.

Jopson, N.B., McEwan, J.C., Dodds, K.G. and Young, M.J. 1995. Economic benefits of including computed tomography measurements in sheep breeding programmes. *Proceedings of the Australian Association of Animal Breeding and Genetics* **11**: 194-197.

Jopson, N.B., McEwan, J.C., Fennessy, P.F., Dodds, K.G., Nicoll, G.B. and Wade, C.M. 1997. Economic benefits of including computer tomography measurements in a large terminal sire breeding programme. *Proceedings of the Association for the Advancement of Animal Breeding and Genetics* **12**: 72-76.

Jopson, N.B., Nicoll, G.B., Stevenson-Barry, J.M., Duncan, S., Greer, G.J., Bain, W.E., Gerrard, E.M., Glass, B.C., Broad, T.E. and McEwan, J.C. 2001. Mode of inheritance and effects on meat quality of the rib-eye muscling (REM) QTL in sheep. *Proceedings of the Association for the Advancement of Animal Breeding and Genetics* **14**: 111–114.

Karamichou, E., Merrell, B.G., Murray, W.A., Simm, G. and Bishop, S.C. 2007. Selection for carcass quality in hill sheep measured by X-ray computer tomography. *Animal* **1**: 3-11.

Karamichou, E., Richardson, R.I., Nute, G.R., Gibson, K.P. and Bishop, S.C. 2006a. Genetic analyses and quantitative trait loci detection, using a partial genome scan, for intramuscular fatty acid composition in Scottish Blackface sheep. *Journal of Animal Science* **84**: 3228-3238.

Karamichou, E., Richardson, R.I., Nute, G.R., McLean, K.A. and Bishop, S.C. 2006b. Genetic analysis of carcass composition, as assessed by X-ray computer tomography, and meat quality traits in Scottish Blackface sheep. *Animal Science* **82**: 151-162.

Karamichou, E., Richardson, R.I., Nute, G.R., McLean, K.A. and Bishop, S.C. 2006c. A partial genome scan to map quantitative trait loci for carcass composition, as assessed by X-ray computer tomography, and meat quality traits in Scottish Blackface Sheep. *Animal Science* **82**: 301-309.

Kempster, A.J., Cook, G.L. and Grantley-Smith, M. 1986. National estimates of body composition of British cattle, sheep and pigs with special reference to trends in fatness: a review. *Meat Science* **17**: 107-138.

Kempster, A.J., Cuthbertson, A. and Harrington, G. 1982. *Carcase evaluation in livestock breeding, production and marketing.* Granada Publishing Ltd, London.

Kirton, A.H. 1989. Principles of classification and grading. In *Meat Production and Processing.* New Zealand Society of Animal Production – Occasional Publication 11. pp. 143–157.

Koerhuis, A.N.M. and Hill, W.G. 1996. Predicted response in food conversion ratio for growth by selecting on the ratio or on linear component traits, in a (sequential) selection programme. *British Poultry Science* **37**: 317–327.

Koohmaraie, M., Kent, M.P., Shackelford, S.D., Veiseth, E. and Wheeler, T.L. 2002. Meat tenderness and muscle growth: Is there any relationship? *Meat Science* **62**: 345–352.

Kukowski, A.C., Maddock, R.J., Wulf, D.M., Fausti, S.W. and Taylor, G.L. 2005. Evaluating consumer acceptability and willingness to pay for various beef chuck muscles. *Journal of Animal Science* **83**: 2605–2610.

Kvame, T. 2005. *Selection for slaughter quality in two genetic lines of sheep by the use of*

computer tomography. PhD Thesis. Norwegian University of Life Sciences.

Kvame, T., McEwan, J.C., Amer, P.R. and Jopson, N.B. 2004. Economic benefits in selection for weight and composition of lambs cuts predicted by computer tomography. *Livestock Production Science* **90**: 123-133.

Kvame, T. and Vangen, O. 2006. *In-vivo* composition of carcass regions in lambs of two genetic lines, and selection of CT positions for estimation of each region. *Small Ruminant Research* **66**: 201–208.

Lambe, N.R., Conington, J., McLean, K.A., Navajas, E.A., Fisher, A.V. and Bünger, L. 2006. *In vivo* prediction of internal fat weight in Scottish Blackface lambs, using computer tomography. *Journal of Animal Breeding and Genetics* **123**: 105–113.

Lambe, N.R. and Simm, G. 2004. Animal breeding and genetics: Traditional animal breeding. In *Encyclopedia of Meat Sciences* (eds. Jensen, W.K., Devine, C. and Dikeman, D), pp. 11-19. Elsevier: Oxford, Volume 1.

Laville, E., Boiux, J., Sayd, T., Bibé, B., Elsen, J.M., Larzul, C., Eychenne, F., Marcq, F. and Georges, M. 2004. Effects of a quantitative trait locus for muscle hypertrophy from Belgium Texel sheep on carcass conformation and muscularity. *Journal of Animal Science* **82**: 3128-3137.

Leymaster, K.A., Shackelford, S.D., Wheeler, T.L. and Koohmaraie, M. 2006. Direct breed effects on growth, carcass and meat quality traits of sheep. *Proceedings of the 8th World Congress of Genetics Applied to Livestock Production*, CD-ROM communication n°. 32-12.

Macfarlane, J.M. 2006. *Growth, development and carcass quality in meat sheep and the use of CT scanning as a tool for selection.* PhD Thesis. The University of Edinburgh.

Macfarlane, J.M., Emmans, G.E., Lewis, R.M., Young, M.J. and Simm, G. 2006. Predicting carcass composition of terminal sire sheep using X-ray computed tomography. *Animal Science* **82**: 289-300.

Macfarlane, J.M., Young, M.J., Lewis, R.M., Emmans, G.C. and Simm, G. 2005. Using X-ray computed tomography to predict intramuscular fat content in sheep. *Proceedings of the 56th Annual Meeting of the European Association for Animal Production*: 264.

Mann, A.D., Young, M.J., Glasbey, C.A. and McLean, K.A. 2005. *STAR: Sheep Tomogram Analysis Routines (version 3.8).* BioSS software documentation, University of Edinburgh.

Meuwissen, T.H.E. and Goddard M.E., 1996. The use of marker haplotypes in animal breeding schemes. *Genetics Selection Evolution* **28**: 161-176.

MLC, 2002. *Sheep Year Book 2002*. Meat and Livestock Commission, Milton Keynes, UK.

Navajas, E., Glasbey, C.A., McLean, K.A., Bünger, L. and Simm, G. 2004. Comparison of manual and automatic segmentation of muscle regions in spiral computed tomography images of sheep. *Proceedings of the British Society of Animal Science* **2004**: 35.

Navajas, E.A., Glasbey, C.A., McLean, K.A., Fisher, A.V., Charteris, A.J.L., Lambe, N.R., Bünger, L. and Simm, G. 2006a. *In vivo* measurements of muscle volume by automatic image analysis of spiral computed tomography scans. *Animal Science* **82**: 545–553.

Navajas, E.A., Lambe, N.R., McLean, K.A., Glasbey C.A., Fisher, A.V., Charteris, A.J.L., Bünger, L. and Simm, G. 2007. Accuracy of *in vivo* muscularity indices measured by Computed Tomography and their association with carcass quality in lambs. *Meat Science* **75**: 533-542.

Navajas, E.A., Lambe, N.R., Sawalha, R.M., Bünger, L. and Simm, G. 2006b. Genetic parameters of *in vivo* muscularity in two divergent sheep breeds: preliminary results. *Proceedings of the 8th World Congress of Genetics Applied to Livestock Production*, CD-ROM communication n° 13-13.

Navajas, E.A. and Simm, G. 2004. Animal breeding and genetics: DNA markers and marker-assisted selection. In *Encyclopedia of Meat Sciences* (eds. Jensen, W.K., Devine, C. and Dikeman, D), pp. 19-27. Elsevier: Oxford, Volume 1.

Nicoll, G.B, Jopson, N.B. and McEwan, J.C. 2002. Contribution of CT scanning to genetic improvement in a terminal sire sheep breeding programme. *Proceedings of the 7th World Congress of Genetics Applied to Livestock Production*, CD-ROM communication n° 11-30.

Nieuwhof, G.J. 2004. A non-linear index to select genetically lean sheep with sufficient fat cover. *Proceedings of the British Society of Animal Science* **2004**: 23.

Nord, R.H. and Payne, R.K. 1995. A new equation set for converting body density to percent body fat. *Asia Pacific Journal of Clinical Nutrition* **4**: 177-179.

ONS, 2006. Annual Abstract of Statistics No 142. National Office for Statistics. http://www.statistics.gov.uk/downloads/theme_compendia/Aa2006/AnnualAbstract2006complete.pdf.

Payne, R.C., Hutchinson, J.R., Robilliard, J.J., Smith, N.C. and Wilson, A.M. 2005. Functional specialisation of pelvic limb anatomy in horses (Equus caballus). *Journal of Anatomy* **206**: 557-574.

Payne, R.W., Murray, D.A., Harding, S.A., Baird, D.B. and Soutar, D.M. 2006. *GenStat for Windows (9th Edition) Introduction.* VSN International, Hemel Hempstead.

Pollott, G.E., Guy, D.R. and Croston, D. 1994 Genetic parameters of lamb carcass characteristics at three end-points: fat level, age and weight. *Animal Production* **58**: 65-75.

Purchas, R.W., Davies, A.S. and Abdullah, A.Y. 1991. An objective measure of muscularity: changes with animal growth and differences between genetic lines of Southdown sheep. *Meat Science* **30**: 81-94.

Purchas, R.W. and Wilkin, G. H. 1995. Characteristics of lamb carcasses of contrasting subjective muscularity. *Meat Science* **41**: 357-368.

Rasch, D., Herrendörfer, G., Bock, J. and Busch, K. 1978. *Verfahrensbibliothek versuchsplanung und -auswertung – Band 2.* Berlin: VEB Deutscher Landwirtschaftsverlag.

Roberts, N., Cruz-Orive, L.M., Reid, N.M.K., Brodie, D.A., Bourne, M. and Edwards, R.H.T. 1993. Unbiased estimation of human body composition by the Cavalieri method using magnetic resonance imaging. *Journal of Microscopy* **171**: 239-253.

Rousset-Akrim, S., Young, O. A. and Berdague, J. –L. 1997. Diet and growth effects in panel assessment of sheepmeat odour and flavour. *Meat Science* **45**: 169–181.

Safari, E., Fogarty, N.M., Ferrier, G.R., Hopkins, L.D. and Gilmour, A. 2001. Diverse lamb genotypes. 3. Eating quality and relationship between its objective measurement and sensory assessment. *Meat Science* **57**: 153–159.

Safari, E., Fogarty, N.M. and Gilmour, A.R. 2005. A review of genetic parameter estimates for wool, growth, meat and reproduction traits in sheep. *Livestock Production Science* **92**: 271-289.

Sañudo, C., Nute, G.R., Campo, M.M., Maria, G., Baker, A., Sierra, I., Enser, M. and Wood, J.D. 1998a. Assessment of commercial lamb meat quality by British and Spanish taste panels. *Meat Science* **48**: 91–100.

Sañudo, C., Sanchez, A. and Alfonso, M. 1998b. Small ruminant production systems and factors affecting lamb meat quality. *Meat Science* **49**: S29-S64.

Savell, J.W. and Cross, H.R. 1988. The role of fat in the palatability of beef, pork and lamb. In *Designing Foods: Animal product options in the marketplace* (ed. Committee on Technological Options to Improve the Nutritional Attributes of Animal Products), pp. 345-355. National Academy Press, Washington, DC.

Schwab, C.R., Baas, T.J., Stalder, K.J. and Mabry, J.W. 2006. Effect of long-term selection for increased leanness on meat and eating quality traits in Duroc swine. *Journal of Animal Science* **84**: 1577-1583.

Simm, G. 1992. Selection for lean meat production in sheep. In *Progress in sheep and goat research* (ed. Speedy A.W.), pp. 193-216.CAB International, Wallingford, Oxon, UK.

Simm, G. 1998. *Genetic improvement of cattle and sheep.* Farming Press, Miller Freeman UK Ltd, Wharfedale Road, Ipswich, UK.

Simm, G., Bünger, L., Navajas, E.A., Lambe, N.R. and Villanueva, B. 2006 Including meat quality in sheep breeding programmes. In *New Developments in Sheepmeat Quality*, 9th Annual Langford Food Industry Conference, pp. 50-54. University of Bristol.

Simm, G., Bünger, L., Villanueva, B. and Hill, W.G. 2005. Limits to yield of farm species: genetic improvement of livestock. In *Yields of farmed species. Constraints and opportunities in the 21st century* (eds Sylvester-Bradley, R. and Wiseman, J.), pp. 123-141. Nottingham University Press.

Simm, G. and Dingwall, W.S. 1989. Selection indices for lean meat production in sheep. *Livestock Production Science* **21**: 223-233.

Simm, G., Lewis, R.M., Collins, J.E. and Nieuwhof, G.J. 2001. Use of sire referencing schemes to select for improved carcass composition in sheep. *Journal of Animal Science* **79**:

E255-E259.

Simm, G., Lewis, R.M., Grundy, B. and Dingwall, W.S. 2002. Responses to selection for lean growth in sheep. *Animal Science* **74**: 39-50.

Simm, G. and Murphy, S.V. 1996. The effects of selection for lean growth in Suffolk sires on the saleable meat yield of their crossbred progeny. *Animal Science* **62**: 255-263.

Szabo, C.S., Babinszky, L., Verstegen, M.W.A., Vangen, O., Jansman, A.J.M. and Kanis, E. 1999. The application of digital imaging techniques in the *in vivo* estimation of the body composition of pigs: a review. *Livestock Production Science* **60**: 1-11.

Thompson, J. M. and Ball, A. J. 1997. Genetics of Meat Quality. In *The genetics of sheep* (eds Piper, L. and Ruvinsky, A.), pp. 523-538. CAB International, Oxon.

Thompson, J.M. and Kinghorn, B.P. 1992. Catman - A program to measure Cat-Scans for prediction of body composition in live animals. *Proceedings of the Australian Association of Animal Breeding and Genetics* **10**: 560-564.

Van Heelsum, A.M., Lewis, R.M., Davies, M.H. and Haresign, W. 2006. Genetic relationships among objectively and subjectively assessed traits measured on crossbred (Mule) lambs. *Animal Science* **82**: 141-149.

Van Heelsum, A.M., Lewis, R.M., Haresign, W. Williams, S.P. and Davies, M.H. 2001. Non-normality in carcass quality measurements and effects on the genetic evaluation of sheep. *Livestock Production Science* **69**: 113-127.

Wade, C.M. and James, J.W. 1996. Optimal allocation of resources considering two sexes and selection in two stages. *Genetics Selection Evolution* **28**: 3-21.

Waldron, D.F., Clarke, J.N., Rae, A.L., Kirton, A.H. and Bennett, G. L. 1992. Genetic and phenotypic parameter estimates for selection to improve lamb carcass traits. *New Zealand Journal of Agricultural Research* **35**: 287–298.

Walling, G.A., Visscher, P.M., Wilson, A.D., McTeir, B.L., Simm, G. and Bishop, S.C. 2004. Mapping of quantitative trait loci for growth and carcass traits in commercial sheep populations. *Journal of Animal Science* **82**: 2234-2245.

Wegener, O.H. 1993. *Whole body computed tomography*. Blackwell Scientific Publications.

Wolf, B.T., Jones, D.A. and Owen, M. G. 2001. Carcass composition, conformation and muscularity in Texel lambs of different breeding history, sex and leg shape score. *Animal Science* **72**: 465-475.

Wolf, B.T., Jones, D.A. and Owen, M. G. 2006. *In vivo* predictions of carcass composition and muscularity in purebred Texel lambs. *Meat Science* **74**: 416–423.

Wolf, B.T., Smith, C., King, J.W.B. and Nicholson, D. 1981. Genetic parameters of growth and carcass composition in crossbred lambs. *Animal Production* **32**: 1-7.

Wood, J.D. 1990. Carcass fatness and meat quality. In *Reducing fat in meat animals* (Wood, J.D. and Fisher, A.V.), pp. 344-397. Elsevier, London.

Wood, J.D., Enser, M., Fisher, A.V., Nute, G.R., Richardson, R.I. and Sheard, P.R. 1999. Manipulating meat quality and composition. *Proceedings of the Nutrition Society* **58**: 363-370.

Wood, J.D., Richardson, R.I., Nute, G.R., Fisher, A.V., Campo, M.M., Kasapidou E., Sheard, P.R. and Enser, M. 2004. Effects of fatty acids on meat quality: a review. *Meat Science* **66**: 21-32.

Young, M.J., Lewis, R.M., Simm, G., Glasbey, C.A. and McLean, K.A. 2001a. *Incorporating X-ray computer tomography into selection programmes to improve the quality of sheep meat.* Scottish Agricultural College final report for the Department of Environment, Food and Rural Affairs (DEFRA).

Young, M.J., Nsoso, S.J., Logan, C.M. and Beatson, P.R. 1996. Prediction of carcass tissue weight *in vivo* using live weight, ultrasound or X-ray CT measurements. *Proceedings of the New Zealand Society of Animal Production* **56**: 205-211.

Young, M.J., Simm, G. and Glasbey, C.A. 2001b. Computerised tomography for carcass analysis. *Proceedings of the British Society of Animal Science* **2001**: 250-254.

Appendix 1: Animal breeding and genetics:

DNA markers and marker-assisted selection

Navajas, E. A. and Simm, G., 2004.
Encyclopaedia of Meat Sciences, (eds. W. K. Jensen, C. Devine and M. Dikeman).
Elsevier: Oxford, Volume 1, pp 19-27.

1. Introduction

Most economically relevant traits in livestock production systems are under genetic control, which implies that both quantity and quality of the final product can be genetically improved by exploiting the variability within or between breeds. Less emphasis has been given in selection schemes in livestock species to those attributes related to carcass composition, and in particular meat quality, than has been given to growth performance.

In the last few decades, more attention has been given to consumers as active participants in the industry. As a consequence of the stronger influence that consumer satisfaction has on the supply chain, increased effort is being directed towards the genetic improvement of carcass and meat quality.

Advances in molecular genetics are leading to valuable applications in the meat industries, such as providing accurate tests of parentage or certifying the origin of specific products (see Carcass identification and traceability). Another relevant contribution is in overcoming the limitations to identifying superior genotypes for meat quality, as a result of the difficulties of collecting phenotypic data on these traits (see Animal breeding and genetics: Traditional animal breeding). Currently, breeding animals can be evaluated for carcass composition by sib progeny testing or indirectly by ultrasound, computed tomography or similar techniques, whereas the evaluation of meat quality is limited to sib or progeny testing. This article focuses on current and possible future detection of genetic markers for carcass and meat quality traits in pigs, cattle and sheep, as well as on how this knowledge can be applied to genetic improvement.

2. Genetic markers, quantitative trait loci and major genes

Most of the relevant carcass and meat quality characteristics are quantitative traits whose phenotypic expression is the result of the joint action of several genes and environmental effects. In general, the evaluation of the genetic merit of individuals and breeds is based on the analysis of phenotypic records plus pedigree information.

Although phenotype is not a perfect predictor of the breeding value, conventional animal breeding methodologies have been effective in the genetic improvement of traits under selection. Successful methods to estimate genetic merit have been developed and applied in breeding programmes (see Animal breeding and genetics: Traditional animal breeding).

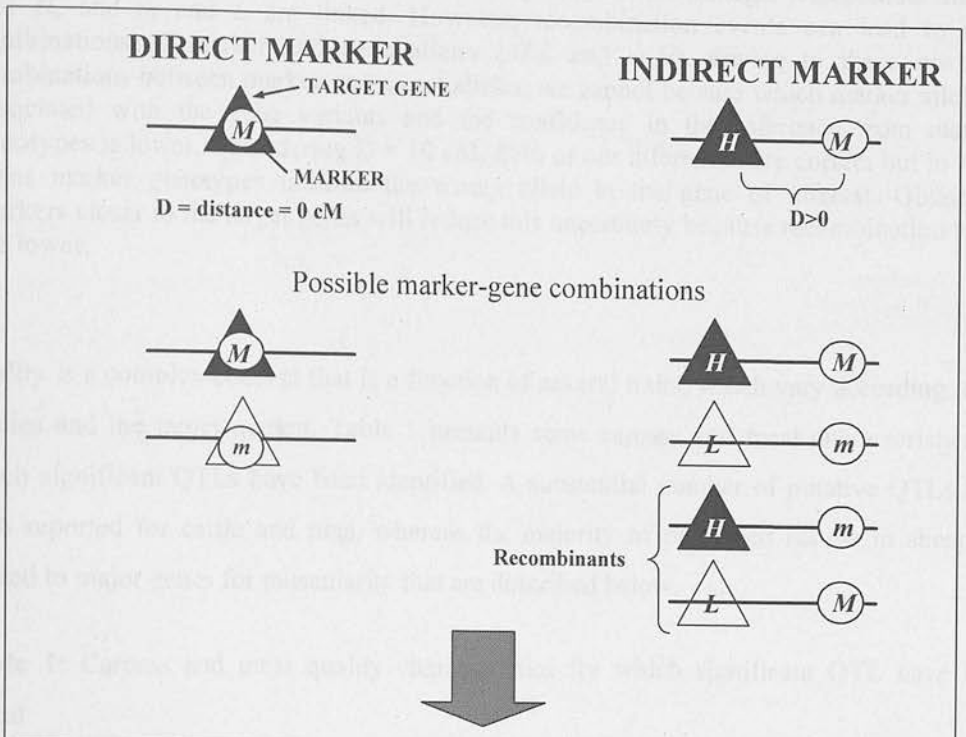
Nevertheless, carcass traits and meat quality are two important groups of characteristics that have not been widely emphasised in breeding programmes because of the difficulties of data collection. The limitation to obtaining phenotypic information is a significant restriction, especially for the genetic improvement of meat quality. Ultrasound, computed tomography and other non-invasive techniques allow the inclusion of carcass composition in the selection schemes using measurements on breeding animals. Although intramuscular fat is considered as an indicator of meat quality and can be measured *in vivo* with moderate accuracy by some of these techniques, there are no methods to directly evaluate other attributes of meat, such as tenderness or ultimate pH, in live animals. The lack of techniques to estimate these characteristics directly in the selection candidates implies that progeny tests have to be implemented if carcass and meat quality are included in the breeding objective. This leads to longer generation intervals and, consequently, slower genetic progress.

As a result of important advances in molecular genetics in the past decade, it is possible to assess variability directly at a DNA level, by using genetic markers. They are segments of DNA with a known position in the genome, which can be identified by laboratory tests (genotyping). By analysing DNA samples that are very easy to extract from tissues samples, i.e. hair, blood or saliva, we can detect which variant (allele) of each genetic marker an animal carries.

In some cases, the marker is the gene of interest, or it may be a fragment of DNA within the gene. If this is the case, knowledge of the marker variant directly indicates which is the gene's variant (direct marker) (Figure 1). However, in most cases genetic markers are non-functional or neutral genes, which are linked to the gene of interest (indirect marker). Despite being non-functional, indirect genetic markers can provide valuable information not only for the identification of the target gene but also for selection on the trait of interest. Direct and indirect genetic markers can be used in genetic improvement schemes, although they require different strategies and lead to different responses to marker-assisted breeding, as will be discussed below.

Genetic markers have become strategic tools for the identification of loci underlying the expression of quantitative traits, known as quantitative trait loci (QTLs). Methodologies used in genetic improvement are based on the infinitesimal model, which assumes that quantitative characteristics are controlled by an infinite number of genes, each with infinitesimal effect. Although quantitative traits are affected by large number of genes (i.e.

they are polygenic), studies performed in the last decade have identified genes for several characteristics that have moderate effects. Important progress has been made in the identification and location of QTLs affecting traits that are economically relevant for livestock production systems, including carcass and meat quality attributes.



Marker genotype	Inferences on genotype with Direct marker	Indirect marker (D=10 cM)
MM	*HH* ✓	*HH* ✓ (20.0%) *HL* × (4.50%) *LL* × (0.25%)
Mm	*HL* ✓	*HH* × (4.50%) *HL* ✓ (41.0%) *LH* × (0.50%) *LL* × (4.50%)
mm	*LL* ✓	*HH* × (0.25%) *HL* × (4.50%) *LL* ✓ (20.0%)
Total 4	**100%**	**81%**

Figure 1: Direct and indirect genetic markers

When a direct marker is available for the gene of interest, the marker variants indicate precisely the allele of the target gene. The genotypes for the genes of interest can be

inferred directly from the marker genotypes. In this example, the markers alleles *M* and *m* are associated with the high (*H*) and low (*L*) performance alleles, respectively. Therefore, a *MM* genotype in a direct marker indicates accurately that the animal is homozygous for the favourable allele (*HH*), whereas *Mn* and *mm* imply that the genotypes for the gene of interest are HL and LL, respectively.

An indirect marker is located close to the gene but it is not the gene itself. The distance D between marker and gene will determine the magnitude of the linkage. We consider that *M* and *H*, and *m* and *L* are linked. However, recombination events can lead to new combinations of marker and gene alleles (*M-L* and *m-H*). Owing to these possible combinations between marker and target alleles, we cannot be sure which marker allele is associated with the gene variants and the confidence in the inference from marker genotypes is lower. Considering D = 10 cM, 81% of our inferences are correct but in 19% cases marker genotypes indicate the wrong allele in the gene of interest. Obtaining markers closer to the target genes will reduce this uncertainty because recombination rates are lower.

Quality is a complex concept that is a function of several traits, which vary according to the species and the target market. Table 1 presents some carcass and meat characteristics for which significant QTLs have been identified. A substantial number of putative QTLs have been reported for cattle and pigs, whereas the majority of published results in sheep are related to major genes for muscularity that are described below.

Table 1: Carcass and meat quality characteristics for which significant QTL have been found

	BEEF CATTLE	PIGS
Carcass Quality	Carcass weight	Carcass length
	Dressing percentage	Dressing percentage
	Predicted saleable beef yield (or retail product yield)	Proportion of lean
	Eye muscle area	Fatness
	Rump (P8) fat depth	Backfat
Meat Quality	Marbling score	Ultimate pH
	Tenderness	Colour
		Water-holding capacity
		Intramuscular fat

The gene of interest may be a single gene with a large effect, named a major gene. Examples of major genes influencing carcass composition and meat quality in pigs, cattle and sheep are presented in Table 2. Note that the influences of these major genes are summarised in the previous article on Traditional Animal Breeding.

The *halothane sensitivity gene* (*HAL*) is one of the best-documented major genes affecting meat quality. Its mode of inherence was established in the 70's (HAL^N, normal, dominant; HAL^n, halothane sensitivity, recessive). It is responsible for the pale, soft and exudative (PSE) meat condition that is associated with a fast rate of post-mortem decline of pH and which is more likely in stress-susceptible animals (porcine stress syndrome, PSS). Homozygous animals (ie those carrying two copies of HAL^n) can be detected by exposure to halothane gas, and this test was used in commercial breeding schemes to select against the recessive gene. Subsequent studies identified the associations between five blood genetic markers and *HAL* alleles. By blood typing, it was possible to identify heterozygotes, which was impossible based only on the halothane test. However, a more accurate identification of the three genotypes is now possible using a direct marker test developed after the identification of the gene (ryanodine receptor gene, *RYR1*) and the discovery of the specific mutation. The high accuracy of this test has led to its broad utilisation by pig breeding companies, with the consequent reduction of losses due to PSS, and the enhancement of pork quality.

Table 2: Some major genes affecting carcass and meat quality

Trait	Locus	Species	Chromosome	Identified gene (mutation)
Muscularity	Callipyge	Sheep	18	--
	Carwell	Sheep	18	--
	Double-muscling	Cattle	2	Myostatin
Meat quality	Halothane (*HAL*)	Pigs	6	*RYR1*
	Rendement Napole (*RN*)	Pigs	15	*PRKAG3*
Intramuscular fat	Adipose Fatty Acid Binding Protein	Pigs	4	*FABP3*
	Heart Fatty Acid Binding Protein	Pigs	6	*FABP4*

The Acid gene or *Napole* gene is another major gene affecting carcass composition and pork quality, especially in Hampshire pigs. The *RN* allele has a favourable effect on carcass length and leanness but increases muscle glycogen content by 70% and is, therefore, responsible for very low muscle pH 24 hours after slaughter (ultimate pH). It is also related to lower yield of cooked ham, which was traditionally assessed using a test known as the Napole Yield. Although the lower ultimate pH may be associated with increase tenderness, it is also related to greater drip loss and paler lean colour. In summary, the *RN* gene, like *HAL*, has a favourable effect on carcass composition but a detrimental influence on meat quality. It has been postulated that the frequencies of both genes increased as a consequence of intense

selection over the last 30 years in the pig industry for growth, efficiency and leaner carcasses.

In sheep, the *callipyge* gene has a pronounced effect on leg muscularity. Characterisation of callipyge lambs indicates greater dressing percentages and heavier and leaner carcasses than in normal lambs. However, the gene also has a severe detrimental effect on tenderness of high-value muscles. The increased toughness of Callipyge meat has been explained by a reduced rate and extent of post-mortem proteolysis that is the result of increased levels of calpastatin. *Carwell* is another gene in sheep that increases muscle development, but in the rib region. Although it has a mild unfavourable effect on tenderness, this is not commercially relevant and may be removed by post-mortem treatment. Both loci map to the same region of chromosome 18, suggesting that they may be alleles of the same gene.

In contrast, the *myostatin* gene in cattle has a favourable effect on both carcass composition and meat quality. This gene is responsible of the double-muscling phenotypes in some European breeds, including Belgian Blue, Charolais and Piedmontese. Meat from double-muscled animals has been evaluated in different studies as quite tender because of reduced collagen content in muscle.

Although the relevance of intramuscular fat content as a determinant of meat quality is controversial, several studies have concluded that it is one of the factors influencing the eating quality of pork and beef. Significant associations between adipocyte and heart fatty acid-binding protein loci (*A-FABP* and *H-FABP*, respectively) and intramuscular fat have been reported. Because of their low effect on backfat deposition, they represent interesting options for improving meat quality through higher contents of intramuscular fat without increasing overall fat levels. In several countries, intramuscular fat content is important economically, even though its relationship with meat palatability is not especially high.

3. Identification of genes and QTL

The increasing understanding of the human and mouse genomes, and the progress in molecular genetic techniques in the last twenty years have been very beneficial for the identification of genes in the main farm species. The recent completion of the human and mouse genome projects, revealing the full DNA-sequences for these species is highly important for the candidate gene approach in farm animals, which is one of the methodologies that have been applied in livestock species to identify significant genes. A

candidate gene is a known gene in a different specie, usually humans or mice, that is related to the physiology underlying the trait of interest. After the candidate gene is chosen, the association between polymorphism/alleles for that gene and the phenotypic expression of the trait is investigated.

A-FABP and *H-FABP* were considered as candidate genes associated with intramuscular fat in the Duroc pig breed. Other candidate gene studies in pigs have focused on myogenin and calpastatin. Their impact on pork tenderness is based on their effect on the muscle fibre number and the rate of tenderisation, respectively (see Conversion of muscle to meat: Ageing).

The *RYR1* gene was identified as a positional candidate gene based on results in human genetics, which indicated that the malignant hyperthemia syndrome in humans was produced by a mutation in this gene. Similarly, the causative gene for the double-muscling condition in beef cattle was found using this approach. While in cattle the gene *mh* was mapped to one end of chromosome 2, studies in mice showed a double-muscling effect of a gene named *myostatin*. This gene, which became the obvious candidate gene for the double-muscling phenotype, was then mapped to a similar position where *mh* was located in the bovine genome. Furthermore, animals of different European breeds that presented double-muscling phenotypes and were homozygous for *mh*, showed mutations that inactivated *myostatin* resulting in increased muscularity.

The other main approach used to search for QTLs and to map major genes in livestock species is termed as genome scan. An extensive list of major DNA-marker trials in farm livestock is presented in Table 3. In genome scans, markers are selected to cover the whole genome, which is examined by looking for regions containing genes of interest. The identification of QTLs is based on the combined analysis of molecular and phenotypic information by searching for significant associations under specific experimental designs. One common design in farm animals is to map QTLs segregating in crosses based on parental populations that are highly divergent for the traits of interest. Figure 2 shows the general concept that underlies the identification of QTLs linked to genetic markers.

Table 3: Genome scans searching for QTL affecting meat and/or carcass attributes

Species	Population	Research group	Country
PIGS †	Large White x Pietrain	Liège University	Belgium
	Meishan x Large White	INRA	France
	Pietrain x (Meishan or Wild Boar)	Hohenheim University	Germany
	Meishan x Large White	Agricultural University of Norway	Norway
	Landrace x Iberian breed	RTA-INIA	Spain
	Wild Boar x Large White	University of Uppsala	Sweden
	Meishan x Large White	Roslin Institute	United Kingdom
	Chinese Breeds x Yorkshire	Iowa State University	United States
	Berkshire x Yorkshire	Iowa State University	United States
	Meishan x Large White	University of Minnesota	United States
	Meishan x Synthetic Line	USDA	United States
BEEF CATTLE ‡	Charolais x Brahman	Cooperative Research Centre	Australia
	Limousin x Jersey	AgResearch- Adelaide University	New Zealand- Australia
	Angus x Brahman	Texas A&M	United States
	(Brahman x Angus) x MARC III	US Meat Animal Research Centre	United States
	(Brahman x Hereford) x MARC III	US Meat Animal Research Centre	United States
	(Piedmontese x Angus) x MARC III	US Meat Animal Research Centre	United States
	Belgian Blue x Marc III	US Meat Animal Research Centre	United States
SHEEP §	Texel Coopworth	AgResearch, Sidney University, Adelaide University	Australia- New Zealand
	Awassi x Merino	Sidney University	Australia
	INRA401	INRA	France
	Fat and Lean selection lines	AgResearch	New Zealand
	Texel, Suffolk and Charollais commercial sire reference animals	Roslin Institute, University of Edinburgh, Scottish Agricultural College	United Kingdom
	Scottish Blackface lean and fat selection lines	Roslin Institute	United Kingdom
	Rambouillet x Romanov	USDA Clay Centre	United States
	Suffolk x Romanov	USDA Clay Centre	United States

Adapted from: † Bidanel and Rothschild (2002); ‡ Burrow *et al.* (2001); § Crawford (2001).

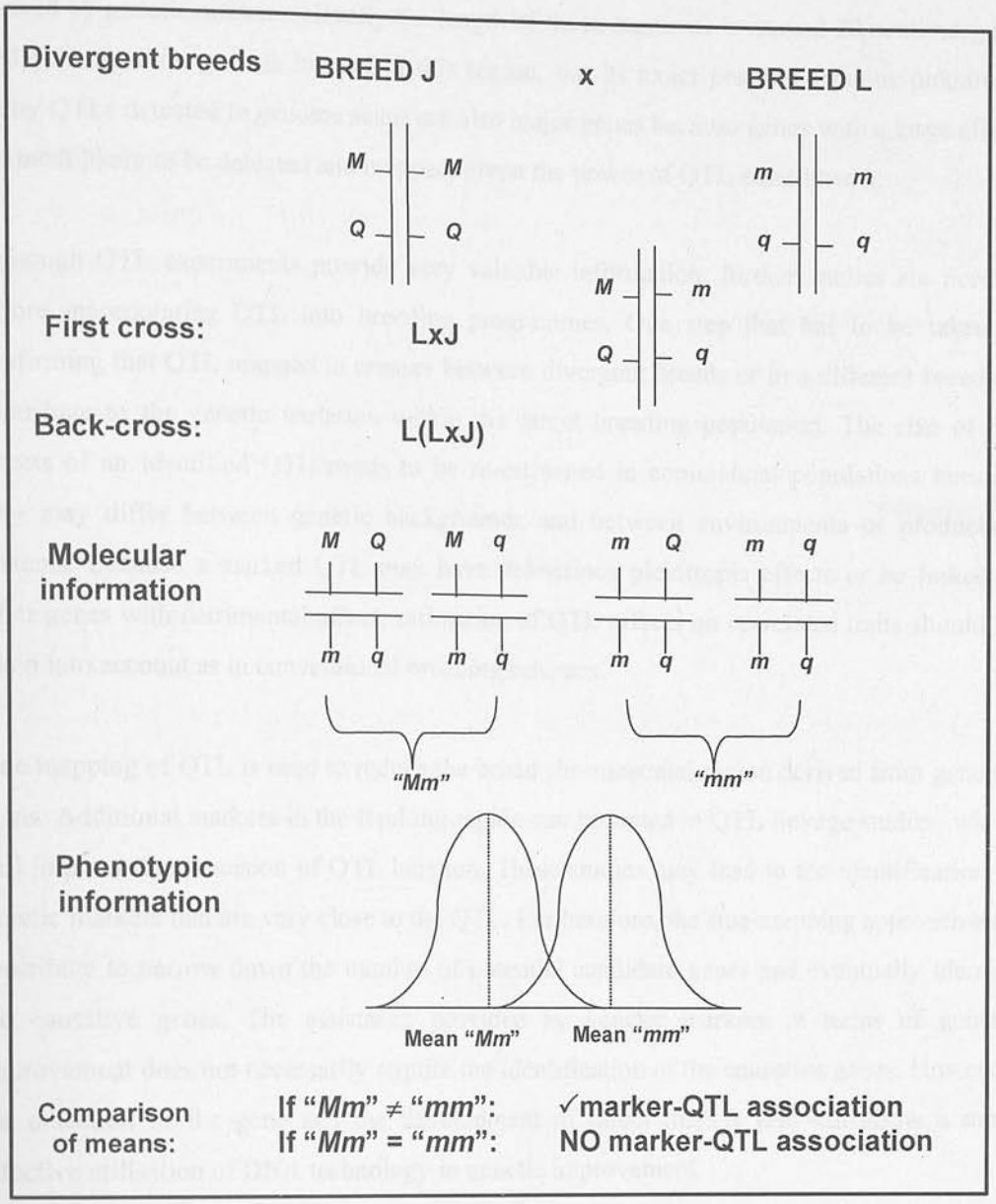


Figure 2: Searching for QTL-genetic marker associations in a back-cross design between two breeds

Breeds L and J are two divergent breeds, which are homozygous for different alleles of both QTL (*Q,q*) and genetic marker (*M, m*). F_1 animals are heterozygous for the marker and linked QTL. Backcrossed animals are obtained by mating F_1 crosses to L individuals. There are four classes of gametes formed by the F_1: the parental gametes *MQ* and *mq* and the recombinant gametes *Mq* and *mQ*. Because parental breeds are homozygous, L gametes are all *mq*. The resulting segregation in the backcross may allow the identification of the QTL. Genotyping provides the information of marker genotypes and phenotypic recording supplies data on the trait of interest. If the average of the *Mm* individuals is significantly different from the average of those with *mm* genotype, it is then concluded that the QTL affecting the trait of interest is linked to the genetic marker.

This approach identifies regions of chromosomes affecting the trait of interest that are

flanked by genetic markers. Usually the length of these segments is around 20 centimorgans (cM); the specific gene is located in this region, but its exact position remains unknown. Many QTLs detected in genome scans are also major genes because genes with a large effect are most likely to be detected and mapped, given the power of QTL experiments.

Although QTL experiments provide very valuable information, further studies are needed before incorporating QTL into breeding programmes. One step that has to be taken is confirming that QTL mapped in crosses between divergent breeds or in a different breed do contribute to the genetic variation within the target breeding population. The size of the effects of an identified QTL needs to be re-estimated in commercial populations because they may differ between genetic backgrounds and between environments or production systems. Because a marked QTL may have deleterious pleiotropic effects or be linked to other genes with detrimental effect, estimation of QTL effects on correlated traits should be taken into account as in conventional breeding schemes.

Fine mapping of QTL is used to reduce the broad chromosomal region derived from genome scans. Additional markers in the flanking region can be tested in QTL linkage studies, which will improve the precision of QTL location. These studies may lead to the identification of genetic markers that are very close to the QTL. Furthermore, the fine-mapping approach may contribute to narrow down the number of potential candidate genes and eventually identify the causative genes. The assistance provided by genetic markers in terms of genetic improvement does not necessarily require the identification of the causative genes. However, the detection of the gene and the development of direct marker test will allow a more effective utilisation of DNA technology in genetic improvement.

An interesting outcome from molecular genetic research is about the inheritance of economically relevant characteristics. Knowledge of mode of action and magnitude of gene effects is relevant to define the strategy to be followed. For example, some genes identified in farm species exhibit non-Mendelian inheritance. The *callipyge* gene, which is associated with muscular hypertrophy and low tenderness of meat in sheep, shows a non-Mendelian inheritance pattern, referred to as polar over-dominance. This pattern implies that only heterozygous individuals having inherited the callipyge mutation from their sires will express the double-muscling phenotype. On the other hand, the mode of inheritance of the *Carwell* gene follows a simple overdominance pattern.

4. Applying the new knowledge in genetic improvement

As was discussed in the previous article on Traditional Animal Breeding, genetic improvement of meat and carcass attributes can be achieved by exploiting the variability within breed and/or using the between-breed differences. In both strategies, genetic markers can play an important role through what is called marker-assisted selection (MAS) and marker-assisted introgression (MAI).

Introgression is the process of integrating superior qualities of one breed (donor breed) into another breed (recipient breed) by crossing the breeds. As illustrated in Figure 3, a first cross between the breeds is then backcrossed to the recipient breed in order to maintain the target gene while eliminating the majority of the genome of donor breed that is inferior for performance in other important traits. Then, animals are intercrossed to achieve homozygosity in the introgressed gene(s).

Markers can be used to select those animals that carry the target gene. Additionally, they can be utilised to accelerate the recovery of the donor genome. Because selection of animals in each step does not rely on observable phenotypes when genetic markers are available, MAI is particularly useful for traits, like carcass and meat quality, that are difficult to measure. However, the effectiveness can be limited when the target characteristic is affected by several genes with moderate effects. Another aspect to be considered is that the genetic gain in other economically important traits is reduced during the introgression. Consequently, it is important to evaluate whether or not the economic benefit of improving meat and/or carcass quality exceeds not only the costs of the programme, but also the losses in response because of genetic lag.

Genetic markers can also be a valuable tool for selecting those animals carrying favourable genes that already exist within a population (MAS). Alternatively, they can be used to eliminate undesirable alleles. MAS, as well as MAI, can be based on direct markers or indirect markers for linked QTLs.

With direct markers, knowledge of marker genotype directly informs us about the QTL genotype (Figure 1). This implies that they can be used to identify superior genotypes without phenotypic or pedigree recording. Nevertheless, sporadic data collection is required to monitor the effects of the target gene in different populations and production systems

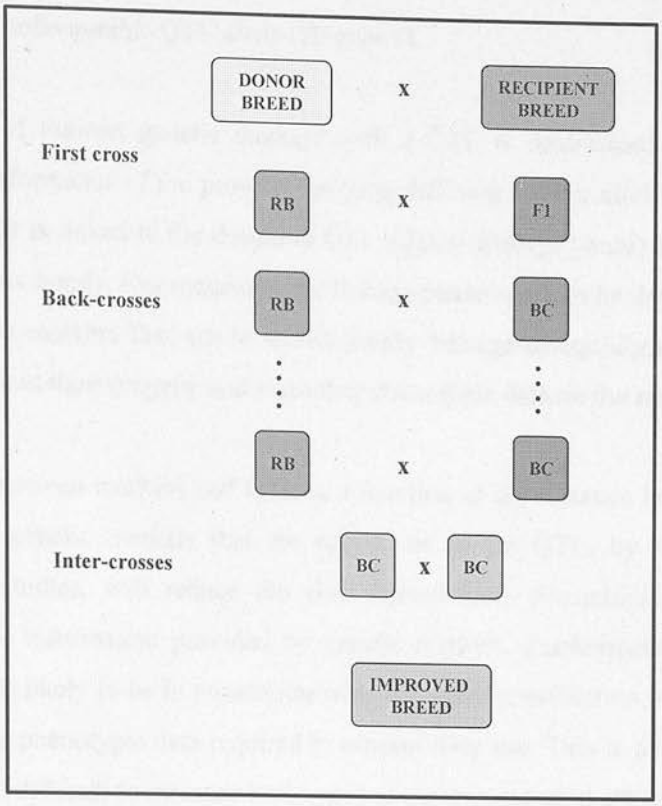


Figure 3: Incorporation of favourable genes from the donor into the recipient breed by introgression
The steps involved in an introgression programme are illustrated. First cross (F_1) individuals are the result of crossing the recipient breed (RB) and the donor population. Backcrossed animals (BC) are produced by mating F_1 and the following generations to the RB. After several generation of backcrossing, crossbred animals are intercrossed to obtain the homozygocity in the introgressed gene(s).

At present, only a few direct markers are available. In general, they are markers for major genes responsible for outstanding phenotypes, in which the existence of the gene was hypothesised before being mapped. Examples are *HAL* and *RN* genes in pigs and *myostatin* in cattle. Although selection against the *HAL* and *RN* genes over the last few decades in the pig industry has been successful, the availability of direct markers has increased its efficacy. In the case of the myostatin gene, different causal mutations in the same gene exist in different cattle breeds. Assays have been developed to detect some of these alleles responsible for commercial use.

Because indirect markers are only near the QTL, the information on the QTL that can be inferred from marker genotypes is not as straightforward as with direct markers. Owing to recombination between loci, markers alleles do not always provide accurate information about the genotype at the linked QTL, and this could mean that animals can be selected

inadvertently for unfavourable QTL alleles (Figure 1).

The association of indirect genetic markers with a QTL is determined by analysing the differences in performance of the progeny carrying different marker alleles. The conclusion about which allele is linked to the desirable QTL alleles (linkage phase) is valid only for a specific sire and its family. Consequently, the linkage phase needs to be determined in all the families for those markers that are in within-family linkage disequilibrium. This requires genotyping sires and their progeny and recording phenotypic data on the progeny.

Recombination between markers and QTL is a function of the distance between them. The identification of genetic markers that are very close to the QTL, by fine mapping and candidate gene studies, will reduce the risk derived from recombination and increase confidence in the information provided by genetic markers. Furthermore, markers tightly linked to QTL are likely to be in population-wide linkage disequilibrium, which will lead to a reduction in the phenotypic data required to support their use. This is particularly relevant for expensive and difficult-to measure traits, such as carcass and meat attributes.

5. Genetic response and cost-effectiveness of MAS

Considerable research has been done on the potential benefits of using MAS in livestock breeding schemes. Studies based on simulations concluded that it is possible to increase genetic gain compared to classical breeding methods by a more accurate estimation of breeding values and the reduction of generation interval.

The utilisation of genetic markers combined with phenotypic records will provide more information on the genetic merit than phenotypic data alone. This can increase the accuracy of selection and consequently the rate of genetic gain. However, this advantage depends on the heritability of the trait. The additional gain from MAS decreases with higher heritability values because phenotypes become better predictors of genotypes and, therefore, genetic markers provide little extra information.

Independent of the heritability, MAS provides great opportunities for the genetic improvement of traits in which phenotypes are difficult to obtain, such as carcass and meat quality traits. Studies have reported predicted increases in responses from MAS using linked markers for these traits in the range of 50 to 60% compared to conventional selection. However, it is important to point out that benefits of MAS has been assessed as the marginal

gain of including molecular data in the actual breeding schemes. Maximisation of MAS benefits may imply the re-optimisation of breeding programmes and changes in breeding strategies.

Although a number of studies have evaluated the rate of genetic gain than can be achieved, only few have investigated economics of including molecular genetics into breeding programmes. MAS can be defined as profitable when the marginal economic gain, which is explained by the genetic progress and the economic value of the trait, overcomes the marginal costs of genotyping and additional phenotyping.

Using linked markers that are in within-family linkage disequilibrium, the implementation of MAS requires molecular information as well as phenotypic data. Consequently, the logistics and the economic merit of MAS are limited, in a similar way to classical selection methods, by the difficulties and high costs of recording phenotypes. On the other hand, the cost effectiveness of MAS is clearer when selection is based on direct markers or alternatively on markers that are tightly linked to QTLs because the requirements for phenotypic recording are lower.

The number of genetic markers in the livestock species has increased and new technologies in molecular genetics are being developed. If a very large numbers of markers along the genome is available, the identification of very close markers linked to QTLs may be achievable in the near future. The research work that is required from a map location obtained from genome scans to the identification of the functional gene is not trivial, although several groups in different countries are dedicated to this objective. Investments of this task seem to be worthwhile for the meat industries because it will lead to a feasible and clearly profitable application of molecular genetics in commercial breeding programmes.

Further readings

Andersson, L. 2001. Genetic dissection of phenotypic diversity in farm animals. *Nature Reviews. Genetics* **2**: 130-138.

Bidanel, J.P. and Rothschild, M. 2002. Current status of quantitative trait locus mapping in pigs. *Pig News and Information* **23**: 39N-53N.

Burrow, H.M., Moore, S.S., Johnston, D.J., Barendse, W. and Bindon, B.M. 2001. Quantitative and molecular genetic influences on properties of beef: a review. *Australian Journal of Experimental Agriculture* **41**: 893-919.

Crawford, A.M. 2001. A review of QTL experiments in sheep. *Proceedings of the Association for the Advancement of Animal Breeding and Genetics* **14**: 33-38.

Dekkers, J.C.M. and Hospital, F. 2002. The use of molecular genetics in the improvement of agricultural populations. *Nature Reviews. Genetics* **3**: 22-32.

Falconer, D.S. and Mackay, T.F.C. 1996. *Introduction to Quantitative Genetics*. 4th edn. Harlow:Logman.

Goodard, M.E. 2003. Animal breeding in the (post-) genomic era. *Animal Science* **76**: 353-365.

Marshall, D.M. 1999. Genetics of Meat Quality. In *The Genetics of Cattle* (eds Fries, R. and Ruvinsky, A.), pp 605-636. Wallingford, Oxon: CAB International.

Sellier, P. 1998. Genetics of meat and carcass traits. In *The Genetics of the Pig* (eds Rothschild, M.F. and Ruvinsky, A.), pp 464-495. Wallingford, Oxon: CAB International.

Simm, G. 1998. *Genetic Improvement of Cattle and Sheep*. Tonbridge: Farming Press.

Speck, P.A., Pitchford, W.S. and Clarke, J.N. 1998. Quantitative and molecular approached to manipulate carcass composition in farm animals: a major quality determinant. *Proceedings of the 6th World Congress on Genetics Applied to Livestock Production*, pp. 149-156.

Thompson, J.M. and Ball, A.J. 1997. Genetics of Meat Quality. In *The Genetics of Sheep* (Piper, L. and Ruvinsky, A.), pp 523-538. Wallingford, Oxon: CAB International.

Appendix 2: Prediction of carcass and joint tissue composition of Scottish Blackface and Texel lambs based on the dissection of the hind legs

1. Introduction

Accurate, full carcass dissection is an expensive and time-consuming procedure. In order to reduce costs and increase the number of carcasses to be included in research trials, different part-carcass dissection techniques have been developed for livestock species (Johnson and Charles, 1981). The complete dissection of a sub-sample of the carcasses and the dissection of just a sample joint in the remaining carcasses is one of the alternatives for carcass composition studies (Connife and Moran, 1972; Cook *et al.*, 1983), which has been applied in different studies in lambs (i.e. van Heelsum *et al.*, 2003) In the latter study, the shoulder was the joint used as predictor of carcass composition.

In the present study, the tissue composition of the hind legs of all carcasses was obtained by dissection with the objective of using this information as predictor of the composition of the whole carcass and other joints. Prediction equations were derived, based on the sample of carcasses that were fully dissected, with the objective of predicting:
(i) the total weight of fat, muscle, and bone in the whole carcass based on the tissue composition of the hind legs, and (ii) the total weight of fat, muscle, and bone in the joints based on the tissue composition of the hind legs.

2. Material and methods

A total of 473 entire male (M) and female (F) Scottish Blackface (SBF) and Texel (TEX) lambs were slaughtered at the University of Bristol.

One hundred carcasses had one side of the carcass fully dissected, whereas in the remaining carcasses only the hind legs were dissected. Carcasses for full dissection were sampled randomly within batch/breed/sire group/sex. Numbers sampled by year, breed, sire group (high and low muscularity, detailed in Chapter 2), and sex are presented in Table 1. Each carcass side (CS) was split into the following joints: thoracic limb (TL), neck and thorax (NT), abdomen (AB), lumbar region (LR) and hind legs (HL). Each joint was then dissected into muscle, subcutaneous fat, intermuscular fat and bone. The absolute weights of the tissue were calculated for each joint and for the side by adding the absolute values of all the joints. The compositional traits included in this study are described in Table 2.

Table 1: Numbers of fully dissected carcasses in 2003 and 2004

BREED/YEAR	HIGH MUSCULARITY			LOW MUSCULARITY			TOTAL
	M	F	Total	M	F	Total	
TEX							
2003	5	6	11	5	6	11	22
2004	7	7	14	7	7	14	28
Total TEX	12	13	25	12	13	25	50
SBF							
2003	6	6	12	5	5	10	22
2004	6	8	14	7	7	14	28
Total SBF	12	14	26	12	12	24	50
TOTAL	24	27	51	24	25	49	100

Table 2: Carcass and joint composition traits and their abbreviations

COMPOSITIONAL TRAITS	CARCASS	JOINTS				
	SIDE	Hind leg	Lumbar region	Abdomen	Thoracic limb	Neck and thorax
Absolute weight						
Weight (W) of joint or carcass	CSW	HLW	LRW	ABW	TLW	NTW
Fat (Subcutaneous fat + intermuscular fat) (F)	F_{CS}	F_{HL}	F_{LR}	F_{AB}	F_{TL}	F_{NT}
Muscle (M)	M_{CS}	M_{HL}	M_{LR}	M_{AB}	M_{TL}	M_{NT}
Bone (B)	B_{CS}	B_{HL}	B_{LR}	--	B_{TL}	B_{NT}

2.1 Data analysis

The prediction equations for tissue absolute weights in the CS and joints were estimated from the composition of the hind legs for each breed independently using GLM (Genstat, 2004). Two models were tested for each these traits.

Model I. Simple regression model $y=\alpha+\beta x$ where α is intercept, β is slope and y and x are the same tissue weight in the CS or joint and HL, respectively (i.e. $y= F_{CS}$ and $x= F_{HL}$).

Model II. Regression equations were fitted for the composition traits using different groups of traits for carcass tissue weights For the prediction of tissue weight in the carcass, Model II included the weights of the three tissues in the HL and HLW, as well as CSW for the prediction of carcass composition or the weight of the corresponding joint. For each trait in the carcass and joints, all possible combinations of predictors (sub-set regression Model II) were fitted for each breed separately, using Genstat (2004). In all cases, the intercepts were fitted. For each regression model, the adjusted R^2 (Adj R^2) and the Mallows coefficient (Cp) as well as the level of significance for each term in the model, were calculated. The best regression models for each trait were selected within the subsets with one, two and three terms. In general, there were problems of co-linearity when more than three terms were included to the model and in some cases when three terms were fitted only.

3. Results and discussion

3.1 Description and comparison of composition of HL and CS

Table 3 presents the averages and standard deviations (s.d.) of cold carcass side and joint weights, as well as the composition of the hind legs of the selected animals and those that had only the HL dissected.

TEX carcasses and HL were heavier than SBF and HL represented a slightly larger proportion of the carcass in this breed compared to SBF (34% *vs* 31%). The proportion of the CSW that corresponds to NT is 3 % higher in SBF compared TEX. The differences between the breeds for the other joints were around 1%.

The composition of the HL in those carcasses selected to derive the prediction equations is very similar to the composition of the remaining carcasses in both breeds. These differences and similarities between breeds are shown in both groups of carcasses in Table 3.

Table 3: Average weights (standard deviation) in kg of carcass side and joints, and weights of fat, muscle and bone in the HL for lambs selected for full dissection and those in which only had HL dissected. Differences between these groups are shown

TEX	Full dissection	With HL dissection only	Difference
CSW	8.25 (1.13)	8.45 (1.13)	2.4%
NTW	3.08 (0.50)	3.17 (0.55)	2.9%
TLW	1.28 (0.15)	1.30 (0.16)	1.6%
ABW	0.44 (0.10)	0.46 (0.10)	4.5%
LRW	0.67 (0.10)	0.68 (0.11)	1.5%
HLW	2.77 (0.36)	2.80 (0.38)	1.1%
F_{HL}	0.30 (0.09)	0.31 (0.10)	3.3%
M_{HL}	2.00 (0.26)	2.02 (0.27)	1.0%
B_{HL}	0.46 (0.06)	0.46 (0.06)	0.0%
SBF	Full dissection	With HL dissection only	Difference
CSW	7.14 (1.14)	7.00 (1.00)	-2.0%
NTW	2.87 (0.51)	2.80 (0.44)	-2.4%
TLW	1.01 (0.12)	1.00 (0.11)	-1.0%
ABW	0.45 (0.12)	0.43 (0.11)	-4.4%
LRW	0.60 (0.12)	0.58 (0.10)	-3.3%
HLW	2.20 (0.31)	2.14 (0.28)	-2.7%
F_{HL}	0.38 (0.12)	0.35 (0.10)	-7.9%
M_{HL}	1.41 (0.19)	1.37 (0.18)	-2.8%
B_{HL}	0.40 (0.05)	0.40 (0.05)	0.0%

Fat, muscle and bone in the HL represented 25/23%, 36/34% and 33/31%, respectively of the total tissue in the CS in TEX/SBF. Similar proportions between breeds were also observed in the other joints. Nevertheless, there were large differences in composition of CS and joints between breeds (Table 4). The smallest difference between breeds was observed in bone (1.2%). SBF had 8.5% higher content of fat and 10% less of muscle than TEX.

Table 4: Averages of observed weights (s.d. in brackets) of fat, muscle and bone in the carcass side and joints of carcasses that were fully dissected (n = 50 in each breed)

	CS	HL	LR	AB	TL	NT
TEX						
F	1.22 (0.44)	0.30 (0.09)	0.06 (0.02)	0.12 (0.06)	0.12 (0.03)	0.63 (0.26)
M	5.53 (0.69)	1.99 (0.26)	0.48 (0.07)	0.32 (0.07)	0.85 (0.10)	1.90 (0.26)
B	1.39 (0.20)	0.46 (0.06)	0.12 (0.03)	--	0.30 (0.04)	0.52 (0.10)
SBF						
F	1.68 (0.60)	0.38 (0.12)	0.10 (0.05)	0.19 (0.09)	0.13 (0.03)	0.88 (0.32)
M	4.09 (0.55)	1.40 (0.18)	0.38 (0.06)	0.25 (0.06)	0.61 (0.07)	1.43 (0.21)
B	1.28 (0.17)	0.40 (0.05)	0.11 (0.03)	--	0.26 (0.03)	0.52 (0.09)

In terms of fatness in the joints, the SBF carcasses were fatter and poorer for muscle in all joints. The maximum and minimum differences in fat between breeds were equivalent to 3.6 and 16% in TL and AB, respectively. The lowest and highest differences in terms of muscle observed in the same joints (6.2 and 16% of the total weight of the joint, respectively).

3.2 Prediction of carcass composition

3.2.1 Prediction of tissue weights based on Model I

The coefficients of determination (R^2), residual standard deviation (r.s.d.), the significance levels (P values) and estimation of the fitted effects for Model I are presented in Table 5.

The weights of the tissues in one side of the carcass can be predicted from the measures in the HL quite well. The accuracies (R^2) are between 77 and 94%, with a tendency for lower values for B_{CS} and higher accuracies in SBF compared to TEX.

Table 5: Prediction of tissue weights based on Model I

	TEX			SBF		
Model I	F_{CS}	M_{CS}	B_{CS}	F_{CS}	M_{CS}	B_{CS}
R^2 (%)	86.6	87.8	77.1	94.2	90.4	79.9
r.s.d.	161	243	96.7	144	170	75.4
P value						
Intercept	0.044	0.025	0.933	0.122	0.408	0.532
Slope	<.001	<.001	<.001	<.001	<.001	<.001
Estimation (standard error)						
Intercept	-167.2	609	9.	-105.0	154	55.5
	(80.9)	(264.)	(108.)	(66.7)	(185)	(88.3)
Slope	4.606	2.468	3.034	4.690	2.795	3.077
	(0.258)	(0.131)	(0.236)	(0.167)	(0.130)	(0.220)
Re- estimation of slope with intercept = 0						
R^2 (%)	--	--	77.6	--	90.4	80.2
r.s.d.	--	--	95.7	--	169	75.0
Estimation	--	--	3.054	--	2.903	3.214
slope (s.e.)			(0.029)		(0.017)	(0.026)

The R^2 values of Model I for the absolute tissue weights were in both breeds higher than for their proportions. Van Heelsum *et al.* (2003) reported a similar trend for 'fat' and 'lean', which was explained as the consequence of the strong effect of the overall size on the weights of muscle and fat. Although these authors predicted the carcass composition from the composition of the shoulder, their reported correlations in crossbred lambs out of Mule ewes were similar to those found in this study (Table 6).

Table 6: Correlations between composition in one joint and carcass side in this study (hind legs) and those reported for Mule wether lambs by van Heelsum *et al.* (2003) (shoulder)

Carcass component	TEX	SBF	Mule
F_{CS}	0.93	0.97	0.95
M_{CS}	0.94	0.95	0.97
$\%F_{CS}$	--	--	0.92
$\%M_{CS}$	--	--	0.85

3.2.2 Prediction of tissue weights based on Model II

For F_{CS} and B_{CS} the best equations for the prediction of tissue weights include the same three variables in both breeds: the relevant tissue weight in the HL, the weight of the joint and the side weight (Table 7). The same traits were also used in the best Model II for M_{CS} in SBF, but not in TEX. For TEX carcasses, F_{HL} replaced the HLW in the best equation for M_{CS}, which suggests that F_{HL} is more informative than HLW in this breed. Regression coefficients for the equations with the highest accuracies are in Table 8.

Table 7: Model II: prediction models for tissue weights (F_{CS}, M_{CS}, B_{CS})

CARCASS SIDE	TEX			SBF		
	Adj R^2	r.s.d	Cp	Adj R^2	r.s.d	Cp
F_{CS}						
CSW	47.04	321	182.69	74.94	299	216.33
CSW + HLW	60.83	276	121.64	75.42	296	207.96
CSW + HLW +F_{HL}	89.06	146	3.26	95.57	126	2.43
M_{CS}						
CSW	83.43	283	197.66	78.48	254	143.54
CSW + HLW	93.14	230	54.74	79.60	132	131.95
CSW + HLW +M_{HL}	94.94	156	29.28	94.23	131	6.73
(CSW +F_{HL}+ M_{HL})	96.78	125	3.43	94.16	132	7.28
B_{CS}						
CSW	72.02	107	28.05	50.22	119	107.54
CSW + HLW	71.60	108	29.60	49.24	120	109.29
CSW + HLW +B_{HL}	81.24	8	5.57	82.96	70	8.36

Note: the models with the highest accuracy were highlighted in this and the following tables.

Table 8: Regression coefficients (standard error) for the best Model II for tissue weights

BREED	TERM	F_{CS}	M_{CS}	B_{CS}
TEX	Constant	5 (171)	8 (146)	-34 (101)
	CSW	0.199 (0.069)	0.578 (0.052)	0.101 (0.033)
	HLW	-0.622 (0.178)	--	-0.141(0.100)
	F_{HL}	4.309 (0.390)	-2.879 (0.392)	--
	M_{HL}	--	0.813 (0.158)	--
	B_{HL}	--	--	2.146 (0.428)
SBF	Constant	-319 (157)	428 (150)	114.7 (90.2)
	CSW	0.288 (0.084)	0.522 (0.089)	0.120 (0.044)
	HLW	-0.703 (0.289)	-2.291 (0.431)	-0.356 (0.162)
	F_{HL}	3.906 (0.266)	--	--
	M_{HL}	--	3.531 (0.322)	--
	B_{HL}	--	--	2.735 (0.282)

3.2.3 Improvement in accuracy between models I and II

The improvements in accuracy were larger in TEX than in SBF, although the average accuracy with Model II, as well as with Model I, was higher in SBF (Table 9). The "ranking" of improvement by trait is similar in both breeds.

Table 9: Accuracies of Model I and best Model II for the prediction of carcass tissue weights

CARCASS	TEX			SBF		
SIDE	Model I	"Best"	Difference	Model I	"Best"	Difference
F_{CS}	86.63	89.06	2.4	94.17	95.57	1.4
M_{CS}	87.81	96.78	9.0	90.36	94.23	3.9
B_{CS}	77.09	81.24	4.2	79.95	82.96	3.0

3.3 Prediction of composition of the joints

3.3.1 Prediction of tissue weights based on Models I and II

Tables 10 to 13 show the accuracies expressed in Adj R^2, r.s.d. and CP of Models I and II for the LR, AB, TL and NT, respectively.

Table 10: Prediction models for tissue weights of LR

LUMBAR REGION	TEX			SBF		
	Adj R^2	r.s.d.	Cp	Adj R^2	r.s.d.	Cp
F_{LR}						
Model I - F_{HL}	65.82	13.8	13.64	79.69	24.0	37.98
Model II						
LRW	30.36	19.7	75.52	71.45	28.4	72.07
LRW + HLW	29.44	19.8	76.55	70.85	28.7	74.02
LRW + F_{HL}	65.95	13.8	14.18	85.73	20.1	13.78
LRW + HLW +F_{HL}	72.81	12.3	3.47	88.57	18.0	3.31
M_{LR}						
Model I - M_{HL}	71.00	38.6	76.00	75.90	30.8	39.06
Model II						
LRW	83.79	28.8	22.18	79.29	28.5	27.08
LRW + HLW	84.77	27.9	18.73	80.37	27.8	23.85
LRW + M_{HL}	87.23	25.6	8.59	85.12	24.2	7.41
LRW + HLW +M_{HL}	88.90	23.9	2.76	86.58	23.0	3.39
B_{LR}						
Model I - B_{HL}	35.03	24.9	25.45	13.61	23.6	48.16
Model II						
LRW	39.12	24.1	20.95	29.54	21.3	30.79
LRW + HLW	42.32	23.5	18.11	44.89	18.8	14.81
LRW + B_{HL}	41.27	23.7	19.25	28.59	21.5	32.21
LRW + HLW +B_{HL}	57.87	20.1	2.40	52.12	17.6	8.81

The Model II with highest accuracy indicates that is possible to predict tissue weights with Adj R^2 ranging from 73% to 93% for F, 75% to 95% for M and 52% to 84% for B. In average, the prediction equations tend to be more accurate in SBF for F (87% *vs* 78%) and in TEX for M (89% *vs* 84%) and B (65% *vs* 61%). The accuracy of F prediction in all the joints was increased by including HLW in the model for the TEX, while in SBF HLW did not improve significantly the accuracy in TL and NT. In both breeds and all joints, the models with weight of the joint, HLW and M_{HL} had the best accuracies, although in the AB and NT the accuracies improved when MHL was replaced by FHL.

The increase in accuracy was more significant in TEX (NT, 4%; AB, 9%) than in SBF (NT, 0.3%; AB, 4%). The highest accuracies were obtained with model II for all tissues and joints. The regression coefficients for the "best" Model II are shown in Table 14, respectively.

Table 11: Prediction models for tissue weights of AB

ABDOMEN	TEX			SBF		
	Adj R^2	r.s.d.	Cp	Adj R^2	r.s.d.	Cp
F_{AB}						
Model I - F_{HL}	67.15	33.0	50.49	81.72	37.9	33.80
Model II						
ABW	60.14	36.3	71.08	78.10	41.5	49.58
ABW + HLW	61.33	35.8	67.21	77.98	41.6	50.11
ABW + F_{HL}	74.45	29.1	29.48	86.96	32.0	11.74
ABW + HLW +F_{HL}	83.25	23.5	5.15	88.97	29.4	4.13
M_{AB}						
Model I - M_{HL}	35.58	52.1	201.28	58.63	58.6	31.86
Model II						
ABW	67.71	36.9	77.94	50.53	41.2	47.09
ABW + HLW	69.10	36.1	72.13	50.54	41.2	47.14
ABW + M_{HL}	72.17	34.3	60.61	61.01	36.6	27.84
ABW + HLW +M_{HL}	79.0.2	29.7	35.17	72.49	30.7	7.61
ABW + HLW +F_{HL}	87.20	23.2	5.07	74.60	29.5	3.81

Table 12: Prediction models for tissue weights of TL

THORACIC LIMB	TEX			SBF		
	Adj R^2	r.s.d.	Cp	Adj R^2	r.s.d.	Cp
F_{TL}						
Model I - F_{HL}	71.16	17.7	5.29	73.88	17.0	9.97
Model II						
TLW	32.43	27.1	74.18	56.21	22.0	47.84
TLW + HLW	31.01	27.4	76.16	61.31	20.7	37.19
TLW + F_{HL}	71.24	17.7	6.09	78.46	15.5	1.19
TLW + HLW +F_{HL}	74.05	2.23	1.24	78.33	15.5	2.50
M_{TL}						
Model I - M_{HL}	71.97	50.4	309.51	64.11	43.3	174.31
Model II						
TLW	92.21	26.6	52.87	89.80	23.1	16.63
TLW + HLW	92.08	26.8	54.42	89.64	23.3	18.30
TLW + M_{HL}	92.54	26.0	48.67	89.78	23.1	17.44
TLW + HLW +M_{HL}	95.10	21.1	17.53	90.56	22.2	13.53
B_{TL}						
Model I - B_{HL}	73.18	20.9	47.63	65.81	19.4	23.59
Model II						
TLW	80.60	17.8	21.73	49.08	23.6	57.62
TLW + HLW	80.97	17.6	21.04	52.57	22.8	50.51
TLW + B_{HL}	83.90	16.2	11.03	67.74	18.8	20.29
TLW + HLW +B_{HL}	83.55	16.4	13.02	75.20	16.5	6.37

Table 13: Prediction models for tissue weights of NT

NECK AND THORAX	TEX			SBF		
	Adj R^2	r.s.d.	Cp	Adj R^2	r.s.d.	Cp
F_{NT}						
Model I - F_{HL}	77.49	122	12.08	90.03	99.6	17.93
Model II						
NTW	57.37	168	64.01	76.50	153	104.65
NTW + HLW	64.21	154	46.43	76.00	155	106.64
NTW + F_{HL}	77.39	122	13.13	92.47	86.6	3.28
NTW + HLW +F_{HL}	81.73	110	3.19	92.78	84.7	2.33
M_{NT}						
Model I - M_{HL}	60.31	167	85.83	67.95	120	44.65
Model II						
NTW	67.59	150	61.65	60.71	133	65.13
NTW + HLW	73.05	137	43.63	60.13	134	66.42
NTW + M_{HL}	75.93	130	34.28	69.78	117	39.69
NTW + HLW +M_{HL}	78.42	123	26.69	81.11	92.2	9.21
NTW + HLW +F_{HL}	85.59	100	3.88	82.46	88.9	5.55
B_{NT}						
Model I - B_{HL}	40.15	79.3	11.53	49.54	64.5	13.81
Model II						
NTW	49.87	72.6	2.19	45.96	67.2	18.05
NTW + HLW	49.77	72.7	3.28	45.19	67.6	19.61
NTW + B_{HL}	51.17	71.6	1.97	57.02	59.9	5.88
NTW + HLW + B_{HL}	50.10	72.4	3.97	58.97	58.5	4.61

Table 14: Regression coefficients (standard error) for the best Model II for tissue weights by joint

TERM		TEX			SBF	
LR	F_{LR}	M_{LR}	B_{LR}	F_{LR}	M_{LR}	B_{LR}
Constant	7.8 (13.9)	-0.8 (26.3)	-14.1 (23.0)	-40.9 (22.5)	12.4 (26.2)	61.3 (22.0)
LRW	0.12 (0.04)	0.57 (0.07)	0.27 (0.06)	0.31 (0.05)	0.39 (0.06)	0.31 (0.05)
HLW	-0.04 (0.01)	-0.12 (0.04)	-0.09 (0.02)	-0.07 (0.02)	-0.10 (0.04)	-0.10 (0.02)
F_{HL}	0.23 (0.03)	--	--	0.31 (0.04)	--	--
M_{HL}	--	0.21 (0.05)	--	--	0.25 (0.05)	--
B_{HL}	--	--	0.42 (0.10)	--	--	0.20 (0.07)
AB	F_{AB}	M_{AB}	B_{AB}	F_{AB}	M_{AB}	B_{AB}
Constant	18.6 (26.7)	-35.4 (26.3)	--	12.4 (39.5)	-24.1 (39.6)	--
ABW	0.31 (0.05)	0.70 (0.05)	--	0.48 (0.08)	0.50 (0.08)	--
HLW	-0.06 (0.01)	0.07 (0.01)	--	-0.10 (0.03)	0.10 (0.03)	--
F_{HL}	0.46 (0.06)	-0.47 (0.06)	--	0.45 (0.07)	-0.45 (0.07)	--
M_{HL}	--	--	--	--	--	--
B_{HL}	--	--	--	--	--	--
TL	F_{TL}	M_{TL}	B_{TL}	F_{TL}	M_{TL}	B_{TL}
Constant	3.2 (21.8)	23.5 (27.0)	-27.2 (20.3)	-22.9 (21.0)	1.0 (29.1)	-14.8 (22.2)
TLW	0.10 (0.04)	0.71 (0.05)	0.17 (0.03)	0.09 (0.03)	0.62 (0.06)	0.18 (0.04)
HLW	-0.04 (0.02)	-0.20 (0.04)	--	--	-0.08 (0.04)	
F_{HL}	0.31 (0.04)	--	--	0.18 (0.03)	--	-0.11 (0.03)
M_{HL}	--	0.24 (0.04)	--	--	0.12 (0.05)	
B_{HL}	--	--	0.24 (0.07)	--	--	0.34 (0.07)
NT	F_{NT}	M_{NT}	B_{NT}	F_{NT}	M_{NT}	B_{NT}
Constant	-26.0 (127)	4.0 (116)	-10.8 (80.2)	-196 (106)	67 (112)	43.0 (76.3)
NTW	0.25 (0.08)	0.57 (0.07)	0.11 (0.03)	0.29 (0.08)	0.47 (0.08)	0.15 (0.05)
HLW	-0.27 (0.08)	0.25 (0.07)	--	-0.21 (0.12)	0.27 (0.13)	-0.16 (0.09)
F_{HL}	2.14 (0.32)	-1.86 (0.28)	--	1.86 (0.18)	-1.46 (0.19)	--
M_{HL}	--	--	--	--	--	--
B_{HL}	--	--	0.41 (0.27)	--	--	0.97 (0.24)

3.3.2 Improvement in accuracy between Models I and II for tissue weights in the joints.

Although Model II improved the accuracy of the prediction of tissue weight in all the joints in both breeds, there are differences between tissues (Table 15). The lowest average increase of Adj R^2 was observed in F for both breeds (TEX, 8%; SBF, 6). The average accuracy of M prediction in TEX had the largest improvement. The results clearly indicate the utilisation of Model II as predictor of tissue weights. Besides the improvements obtained with Model II, the lowest accuracy corresponds to B weights in the LR and NT of both breeds (range between 51 and 60%).

Table 15: Accuracies of Model I and best Model II for tissue weights in the carcass joints

Regio	TEX			SBF		
n	Model I	"Best"	Difference	Model I	"Best"	Difference
LUMBAR REGION						
F	65.82	72.81	7.4	79.69	88.57	8.9
M	71.00	88.90	18.9	75.90	86.58	10.7
B	35.03	57.87	20.9	13.61	52.12	38.5
ABDOMEN						
F	67.15	83.25	16.2	81.72	89.97	8.3
M	35.38	87.20	51.9	58.63	74.60	16.0
B	--	--	--	--	--	--
THORACIC LIMB						
F	71.16	74.05	3.0	73.88	78.46	4.6
M	71.97	95.10	23.4	64.11	90.56	26.5
B	73.18	83.90	11.1	65.81	75.20	9.4
NECK AND THORAX						
F	77.49	81.73	4.3	90.03	92.78	2.8
M	60.31	85.59	25.3	67.95	82.46	14.5
B	40.15	51.17	9.8	49.54	58.97	9.4

4. Conclusions

In summary, more accurate predictions of tissue weights in carcasses and joint were obtained using Model II. In general, Model I had higher accuracies of the carcass composition than in the joints, and less significant improvements were obtained with Model II.

Predictions equations were derived for both breeds that had in general high values of Adj R^2 for both carcass (>81%) and joint compositions (>73%). Lower accuracies (~55%) are only expected in the case of bone weights of TL and NT.

References

Conniffe, D. and Moran, M.A. 1972. Double sampling with regression in comparative

studies of carcass composition. *Biometrics* **28**: 1011-1023.

Cook, G.L., Jones, D.W. and Kempster, A.J. 1983. A note on a simple criterion for choosing among sample joints for use in double sampling. *Animal Production* **36**: 493-495.

Genstat 7 Release 7.2 (PC/Windows NT) 2004. Genstat 7 Committee, Lawes Agricultural Trust (Rothamsted Experimental Station).

Johnson, E.R. and Charles, D.D. 1981. The use of carcass cuts to predict beef carcass composition: a research technique. *Australian Journal of Agricultural Research* **32**: 987-997.

Van Heelsum, A.M., Lewis, R.M., Davies, M.H., and Haresign, W. 2003. Growth and carcass characteristics in wether lambs of a crossbred dam line. *Animal Science* **76**:45-53.